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Studies Concerning Mosaic Diseases

by

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SCOPE OF THE STUDIES

In view of the importance of the pathological histology in mosaic diseases and because of the fact that in no publication to date is there any correlation of the histology in this group, the present writer has accumulated evidence regarding the histological characteristics of diseased plants from as wide a field as possible.

To do this many thousands of plants have been examined and studies have been made of hundreds of sections, both freehand and microtome. No statements are made as to technique in staining, etc., except where other than ordinary procedure was followed.

During the course of the studies further evidences of aphid inoculation were obtained, new mosaic diseases discovered or differentiated and inheritance of mosaic through seed determined, and these facts are incorporated in the paper.

In addition the experiments of Lodewijks concerning the relation of colored light to mosaic were repeated under more exact conditions and furthermore preliminary tests were made to determine the effect of light on the expressed juices of diseased plants. Actually the problem of the relation of light to diseased plants and to the "virus" involves an extended series of physiological studies beyond the scope of the present paper.

INTRODUCTION

During the development of phytopathology marked advances have been made as a result of the study of great groups of diseases, such as for instance those caused by bacteria. Investigators are now faced with a group of baffling diseases of profound interest and rapidly growing economic importance collectively known as "mosaic diseases" and it is within the realm of probability that the determination of the etiology in this group will bring about a similar advance..

The name has arisen from a tobacco disease, which, according to Hunger, was first described by Sweiten in 1857, and named by Adolp Mayer in 1886 "Mosaikkrankheit". In naming it, however, Mayer did not clearly distinguish between "mosaic" proper and another disease known as "Pockenkrankheit". Nevertheless, to Mayer must be ascribed the origin of the name. "Mosaic" is now generally used as the name for those diseases of unknown etiology in which two characteristic symptoms occur. The first is a mottling, unlike variegation or a chimera, due to modifications in the structure and coloration of the chlorenchyma of certain leaf areas. The second is the infectious nature of the sap from diseased plants, even after filtration, although in this sap no mosaic disease-producing organisms have yet been demonstrated.

The term "infectious" is here meant to cover not only natural and artificial transmission of the disease from plant to plant but systemic transmission in the plant.

The work of Jodidi *et al.* (75) would seem to point to a third characteristic, biochemical in nature, since these investigators find that in certain mosaic diseases denitrification takes place whereby nitrates are reduced to nitrites which bring about elimination of nitrogen in free state. Until results from other mosaic diseases corroborate these findings this possible characteristic is merely noted.

Within recent years mosaic has been found in many plants other than tobacco as the following list will show.

SOLANACEAE

Nicotiana tabacum, N. glauca, N. rustica, N. viscosum, N. silvestris, N. longiflora, N. alata, N. langsdorfi, N. plumbaginifolia, N. forgetiana, N. paniculata, Solanum tuberosum, S. nigrum, S. dulcamara, S. carolinense, S. aculeatissimum, Capsicum annuum, Hyoscyamus niger, Datura stramonium, D. fastuosa, D. quercifolia, D. tatula, Petunia violacea, Nicandra physaloides, Physalis longifolia P. subglabrata, P. virginiana, P. heterophylla.

According to Nishimura (100) *Physalis Alkekengi* does not show symptoms of mosaic but is nevertheless an efficient "carrier".

All attempts by the writer to infect *Solanum Melongena, Atropa belladonna, Lycium vulgare* and *Salpiglossis sinuata* have so far failed but Melhus (97) finds that with *S. Melongena* the symptoms are masked after the seedling stage.

CUCURBITACEAE

Lagenaria vulgaris, L. leucantha, Cucurbita pepo, C. moschata, C. maxima, Citrullus vulgaris, Cucumis melo, C. sativus, C. anguria, C. grossulariaeformis, C. metulliferus, Luffa cylindrica, L. acutangula, Micrampelis lobata, Sicyos angulatus, Momordica involucrata, M. charantia, Ecballium elaterinum, Benicasa hispida, and Trichosanthes anguina.

The last five proved susceptible (48) upon artificial inoculation.

Micrampelis (Echinocystis) lobata is common as a wild plant and Doolittle (48) correlates the prevalence of mosaic in cultivated Cucurbitaceae with occurrence of diseased wild cucumber in the same locality.

GRAMINEAE

Saccharum officinarum, Zea Mays, Sorghum vulgare, Panicum dichotomiflorum, Syntherisma sanguinalis, Chætochloa lutescens, Panicum miliaceum.

LEGUMINOSEAE

Lathyrus odoratus, Vicia faba, Trifolium pratense, T. hybridum, T. repens, T. incarnatum, Melilotus alba, M. officinalis, Medi-

cago lupulina, M. arabica, Phaseolus vulgaris, P. lunatus, P. lunatus macrocarpus, P. acutifolius latifolius, Arachis hypogœa, Pisum sativum, Soja max.

ROSACEAE

..*Rubus strigosus,* and cultivated varieties of raspberry, especially Cuthbert and Marlboro.

ASCLEPIADACEAE

Asclepias syriaca.

PHYTOLACCACEAE

Phytolacca decandra.

ANACARDIACEAE

Rhus typhina.

LAURACEAE

Persia gratissima.

CONVOLVULACEAE

Ipomœa Batatas.

PEDALIACEAE

Martynia louisiana.

LOBELIACEAE

Lobelia erinus var gracilis.

CHENOPODIACEAE

Beta vulgaris.
Spinacia oleracea.

Lind (81) states that beet mosaic causes serious losses in the seed crop but that it is never found in sugar beets. It occurs in Denmark chiefly.

Robbins (112), however, describes a mosaic of sugar beet, previously announced by Townsend (149), in which phloem necrosis occurs and in which affected leaves are abnormally thick and brittle with a constant abnormal accumulation of starch. Although the writer has not seen any of this material, the fact that phloem necrosis and abnormal starch accumulation are characteristic symptoms points to it being rather a type of "Curly top" than mosaic.

AMARANTACEAE

Amaranthus retroflexus. It is stated by Doolittle that there is evidence that *A. retroflexus* is susceptible to cucurbit mosaic.

CRUCIFERAE

Brassica pekinensis, B. japonica, B. rapa.

CICHORIACEAE

Lactuca sativa. (Boston Head and Paris White Cos).

AMBROSIACEAE

Ambrosia trifida.

COMPOSITAE

Helianthus debilis.

AMARYLLIDACEAE

Hippeastrum equestre.

SCITAMINACEAE

Canna indica, Musa Cavendishii.

COMMELINACEAE

Commelina nudiflora.

SYMPTOMATOLOGY

It is reasonable to argue that the term "symptom" may include those of the most minute type and therefore under this head we could discuss histological and cytological characteristics of mosaic. I shall, however, include only microscopic symptoms and, following customary procedure, deal with the microscopic under the heading "pathological histology".

GENERAL APPEARANCE OF DISEASED PLANTS

The appearance of diseased plants in the field depends in a large measure on whether they were affected early or not. If they contract the disease in later growth stages only upper parts exhibit symptoms so that there is a similar condition to "mottle-top" in tobacco. The exception to this is potato where no evidences of mosaic may appear until the next season. Various investigators (Allard, Brandes, Schultz, Folsom, etc.) have stated that plants which contract the disease early in life are distinctly paler in general appearance and can, therefore, readily be distinguished from healthy plants in the field. My observations confirm this with tobacco, tomato, potato and sugar cane and show that the same holds true for raspberry, petunia, pepper, Hyoscyamus, sweet pea, Canada field pea, kidney bean, Windsor bean and clovers. The paler appearance is due to reduction of the chlorophyll content and therefore varies with the virulence of infection.

Dwarfing is a general symptom (Allard, Schultz, Folsom, Rosen, Doolittle, etc.) and to a varying extent this is accompanied by spindling growth. In Quebec weak, spindling, rather recumbent stems are frequently a pronounced symptom of mosaic in Green Mountain potatoes while in Cobbler dwarfing due to shortened internodes is typical. The dwarfing is at times concurrent with considerable ruffling and curling of the foliage giving rise to a condition known loosely as "curly dwarf." Quanjer (106) holds the view that a curly dwarf type which he denominates "Welvingszeikte" is an advanced stage of mosaic. From my observations in this province what is commonly known as "curly dwarf" is really a mosaic dwarf since tubers from dwarfed plants give rise to typical severe mosaic when grown in the greenhouse.*

Spindling and somewhat dwarfed habit is usual with raspberry mosaic in turions of the second and subsequent years in marked contrast with the dwarfed but stocky growth of canes affected with "curl".

Canada field peas, especially Golden Vine and Arthur varieties, when diseased develop weak stems which modify the habit of the plants considerably. This is not so noticeable with sweet peas since they are grown under conditions conducing to maximum development. With pea or kidney beans, however, straggling, weak stems and runners are very evident in later growth. Red, crimson, trefoil, white and alsike clovers all show dwarfed growth with weaker stems but with white and yellow sweet clovers these characteristics are not so pronounced or may be entirely absent.

My observations show that accompanying the habit of dwarfed, spindling growth is a tendency to excessive branching in the case of raspberry, tomato, pea bean, Canada field pea, and Green Mountain potatoes.

Premature yellowing and dropping of the lowermost leaves occurs with mosaic of potato, tomato, and beans but with soy bean diseased plants remain green longer than healthy plants (Gardner and Kendrick, 59). I have noticed delayed maturity in the upper leaves of pea beans and potatoes but not in the plant as a whole.

It is an accepted view that premature death of tissues does not generally accompany mosaic even in severely infected cases and the writer's studies support this. With field tomatoes suffering from a particularly virulent infection in 1920 and again in 1921 there was premature death of the hypoplastic, chlorotic areas in the leaves while the dark green parts were still living. There is no doubt that the weaker areas could not live under the dry conditions of summer. In the greenhouse I have not observed any necrosis even where infection was equally severe with that in the field. A similar condition is found in soy bean (Gardner and Kendrick); in *Nicotiana rustica* Allard (1) finds that tissue death is of frequent occurrence; and in the secondary symptoms of mosaic of sugar cane necrotic areas may be found in cane tissues (Brandes, 27). With potatoes a doubtful situation occurs at times for necrotic lesions develop quite suddenly in leaves, petioles and stems of diseased plants, and leaves wilt rapidly hanging limp by a mere thread of tissue or dropping away completely. Such a condition has been named "Streak" (Orton) and "Leaf drop" (Murphy). It cannot be affirmed that streak and leaf drop are cases of tissue death in potato mosaic but the writer has never yet found streak and leaf drop unassociated with mosaic. Because of this fact, it is included here and discussed later in the pathological histology as related to mosaic although more detailed studies are necessary correctly to determine the situation.

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* Since the above was written Krantz and Bisby (Bull. 197, Minn.) advance the same view but Murphy (Bull. 44, Ottawa) inclines to the view that "Crinkle" which to a certain extent resembles curly dwarf and mosaic is a separate disease.

LEAVES

(a) MOTTLING

Under ordinary conditions of infection a mottling in dark and light green areas is the typical symptom. The size and location of the lighter areas vary considerably, as for example in potato where they are often small, angular and scanty, while in tobacco they are larger, less angular and numerous (Pl. I, Figs. 1, 3, 4.) Mottling of this kind is usual with tomato, tobacco, potato, pepper, clovers, Canada field pea, sweet pea, Red kidney bean, Windsor bean, and marrow bean, and it is unaccompanied by distortion or curling. There is a certain amount of dwarfing, and it is possible in a good light to detect the ridges delimiting the dark green areas from the light green caused by the difference in level of the upper epidermis over those areas. Raspberry mosaic late in the season shows a similar mottled effect. With Hippeastrum, corn and sugar cane mosaics the mottling is different only in respect to the rather linear areas into which it falls owing to the nature of the leaves and their venation. (Pl. IV, Figs. 2, 5.).

(b) CURLING AND RUFFLING

When the areas involved, either in dark or lighter green, are larger the leaves tend to more unequal developmnt of the tissues so that considerable ruffling or curling occurs. This is of frequent occurrence on potato, tomato, tobacco, raspberry, pea bean, Canada field pea, clovers, and it is characteristic of lettuce (Jagger), Chinese cabbage, mustard and turnip (Schultz), sweet potato (Rosen), cucumber, etc. (Doolittle), and soy bean (Gardner and Kendrick). In addition to the ruffling there is a constant tendency for the leaves to curl downward at the margin and in the case of potato the curled and ruffled condition may altogether mask the mottling.

(c) SAVOYING OR BLISTERING

Early and probably heavier infection in the case of tomato, tobacco, petunia, black henbane and raspberry involves more of the leaf tissues in the lighter green and the darker green is seen as islands (Pl. I, Figs. 1, 5.). Such islands of darker green are hypertrophied (discussed later) and give the leaf a distorted, blistered appearance. I have found a similar condition in a pole bean (Cluster Wax) and it is usual with pea beans (Pl. II, Fig. 11.) in contrast with Red Kidney and marrow beans. It appears to be common in soy bean as indicated by illustrations (Gardner and Kendrick, 59) and cucumber, etc. (Doolittle). Blistering has been the rule with field tomatoes at Macdonald College and I have never seen other than blistering in the case of petunia and black henbane. Allard's illustrations (5) of petunia mosaic indicate that his findings were identical although this does not preclude the possibility that ordinary mottling occurs.

(d) DWARFING AND DISTORTION

The areas chiefly affected may be marginal or may extend from the midrib to margin when the outline of the leaf becomes undulating and distorted and the leaf extremely asymmetrical. In tobacco I have frequently observed that when the lamina of the apical third of the leaf is most affected the direction of the veins is changed so that they converge towards the tip of the leaf thus changing the leaf appearance entirely and it is not unusual in such cases for enations to develop on the under side. Long, sinuous, ribbon-like leaves are produced by diseased tobacco plants (Allard), and Hunger (66) illustrates a remarkable case of phyllodiniation in which all but one leaf on a plant were merely midribs. Filiform leaf and "fern" leaf have been observed many times in both greenhouse and field on Livingstone Globe and John Baer tomatoes at Macdonald College. "Fern" leaf is reported from Holland (Westerdijk) on tomato while filiform leaf occurs in sweet potato mosaic (Rosen). Distortion and asymmetry are typical in petunia and black henbane and to a lesser extent in pea bean and Cluster Wax bean. Canada field pea leaves are not distorted to so great extent but are wrinkled and asymmetric with a somewhat dissected margin, and no distortion comparable with that in tobaacco, etc., has been found in the clovers. Where distortion occurs it is generally due to more acute and heavier infection in a very susceptible stage, e.g. in the seedling, since drastic changes in metabolism and histology may eventuate under such conditions. It is always possible, however, that cases of phyllodiniation, fern leaf, and the development of enations, may arise as a result of unfavourable environment (Clinton, 34) with or without infection and it is not contended here that these phenomena are symptoms of mosaic so much as symptoms of the extreme effects of mosaic combined with unfavourable environmental conditions.

FLOWERS

(a) Mottling and Distortion

Normally the flowers of tobacco are gamopetalous and spinel pink in colour,* but in diseased plants there may be both mottling and catacorolla. In health the corolla is gamopetalous but with mosaic it is often deeply cleft so that there are pseudo-petals which when filamentous bear ascidia at the tips (Allard, 1). In other instances the corolla is dwarfed and does not open but the pistil and part or all of the stamens protrude. The mottling may be slight, when small white streaks occur, or may present a condition in which only a narrow strip of pink is left in the middle of each corolla lobe.

Mottling with occasional distortion has been noted in petunia (Allard) and I have observed mottling in red purple varieties but no distortion of the corolla has been found. During the summer of 1920 I constantly observed mottling in the flowers of red and purple varieties of diseased sweet pea and similarly in the case of cultivated violet. The latter, although almost certainly mosaic-diseased, has not been incorporated in these studies.

Distortion without mottling is sometimes observable in the blossoms of tomato, beans and peas but has not been noted in raspberry, potato or pepper. Blossoms produced in the later stages of cucumber mosaic are dwarfed and slightly paler than normal blossoms (Doolittle).

(b) Fall of Blossoms

The reduced yield from mosaic-diseased plants is partly accounted for by the fact that many flowers fall prematurely. With tomato, raspberry, sweet pea, pea bean and potato this is often marked and in the case of raspberry and tomato I have counted as high as thirty per cent fall in the field. Usually this is still more pronounced in potato for diseased plants will show the flower stalk with flowerless peduncles, every blossom having fallen prematurely. In the greenhouse blossom fall of tobacco to the extent of twenty per cent has been noted but in the field there is apparently very little. Westerdijk (141) remarks that in Holland a great number of blossoms either blight or drop off before fertilization and Doolittle (48) notes the greatly reduced number of pistillate flowers in diseased cucumbers, etc., although he does not say whether reduction is due to falling or to non-development.

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* Ridgway's Color Standards.

FRUIT

(a) MOTTLING

During the summers of 1920 and 1921 I collected hundreds of tomato fruits which while green were beautifully mottled. The illustration on Plate II, Fig. 7, was made from an ordinary sample showing fruit mottling. The ridges marking the limit of dark green and the sudden depression of the epidermis into the light green may easily be seen and very often are so pronounced that they can be felt. On ripening the ridges remain but gradually the mottled appearance diminishes although the light areas do not redden so rapidly as the dark green. Russetting of diseased fruit is of frequent occurrence but I have not yet been able to determine that such russetting is due to the mosaic disease.

A similar mottling is reported by Westerdijk (141) and McKay (92) illustrates and describes a marked cracking and irregular brownspotting of the fruit. The case cited by McKay is perhaps a more advanced stage of the russetting I have observed but it is so extreme that it is probably due to a combination of circumstances of which mosaic may be one.

I have made many attempts to procure fertilization in potato so that potato "balls" might develop in the hope of finding that they were mottled, and also to test seed inheritance, but have not yet succeeded in any instance.

A quite similar mottling, but less obvious, I have seen on diseased pepper fruits and sometimes on bean pods, especially those of Vicia faba, although usually the bean pods are lighter colored generally and do not show mottling.

The most marked mottling is that in cucumber, Crookneck squash and Large Cheese pumpkin (Doolittle).

(b) DWARFING AND DISTORTION

Tomato fruit in the field, much more than in the greenhouse, are dwarfed and often misshapen as a result of mosaic. In fact it is unusual to obtain full-sized, well-shaped fruit from diseased vines. Peas, Kidney beans and broad beans are dwarfed and broad beans may also be considerably distorted. Soy beans are stunted, flattened, less pubescent and more acutely curved than those on normal plants (Gardner and Kendrick) and with cucumber swellings, some isolated and some merging into large protuberances, give rise to the name "nubbin" or "wart disease" (Doolittle).

(c) REDUCTION IN SEED AND IN SEED VITALITY

Definite information concerning the loss in seed or in the germinating capacity of seed from mosaic-diseased plants is scarce. Brandes (29) tagged diseased and healthy corn plants in Louisiana in May, 1920, and in August out of 20 diseased plants 17 were completely sterile and the other three had a few scattered kernels while healthy plants produced full ears.

With soy beans the yield of seed is materially reduced since a considerable number of pods contain no viable seeds and the others a reduced number (Gardner and Kendrick). These authors (p. 112) planted in one case 150 seeds from diseased plants of which 124 came up and in another 180 seeds of which 106 germinated while apparently controls germinated normally.

There is reduction in set of seed in Quebec with peas and beans but no complete figures are at present available. It should be pointed out that in considering reduction in seed only the cases in which pods or fruit actually form are here dealt with since we are discussing fruit symptoms. Taking into account the non-setting of fruit due to blossom fall, etc., the loss of seed is much heavier. Thus in some cases with pea beans not a single pod will form although there may be profuse flowering.

My counts of 40 healthy and 40 diseased Windsor or broad beans during 1920 showed that the average healthy pod contained 3 seeds whereas the diseased pods contained only 1.3 good seeds.

Reference has later been made to red clover in which 22 healthy plants yielded 10,566 seeds with 75 per cent germination, while 22 diseased plants gave 1,443 seed with only 13 per cent germination. In greenhouse work with Canada field pea, out of 270 seeds from diseased plants sown, 197 germinated of which 6 died soon after germination, while with seed from healthy plants a failure is rare.

It is therefore obvious that in the Leguminoseae there is a distinct reduction in the number of fruit set, of seed in the fruit, and in the germinating power of the seed of mosaic diseased plants.

That such is the case with other plants is practically certain but the fruits of tobacco, tomato, cucumber, etc., contain many seeds and special counts have not been made. With potato no fruit has been available.

STEMS

(a) Mottling or Striping

Since stems usually contain chlorenchyma it is reasonable to expect to find symptoms therein but very few cases are recorded. Westerdijk (141) cites instances of spiral stripings of lighter green on tomato stems, Chapman (32) of mottling of tobacco stems in rare cases, and Doolittle (48) a slight mottling with squash and pumpkin.

During 1920 and 1921 the writer noted a total of 7 tobacco plants with mosaic which showed striping (Pl. VI, Fig. 11) out of some three thousand and during 1921 a total of 15 tomato plants with streaked stems out of approximately two thousand five hundred plants. A more general characteristic of the diseased stems is the lighter tone of green, and not only is this noticeable in tomato and tobacco but it may be seen in potato, peas, and beans. Naturally other conditions will accentuate or modify color in stems but with mosaic diseased plants the lighter green is predominant. In order to see whether potato tubers developed in light would show mottling two series of tests were arranged. In one four plants with well-developed mosaic were grown in large pots and just at the initiation of tuber formation the stolons were removed and the cut ends sealed. Axillary aerial tubers were formed which grew to be an inch by three-quarters in size but no mottling could be seen. With four other well-diseased plants the stolons were carefully uncovered and kept in the light during tuber development. The tubers were smaller than normal as was expected but again there was no mottling.

(b) DWARFING

Stem dwarfing both in internodal length and circumference is usual with potato, tomato, beans, peas, tobacco, petunia, sweet pea, clovers, raspberry, etc., and is also found in mosaic of cucurbits (Doolittle), soy bean (Gardner and Kendrick), corn (Brandes), sugar cane (Brandes, Lyon), and sweet potato (Rosen). Dwarfing is extreme when infection occurs early and severely, and on the contrary in many cases of ordinary mosaic it can barely be determined with certainty.

(c) CANKERING

The work of Brandes (27), Lyon (85), Matz (86) and Kunkel (79) makes it reasonably certain that in corn and sugar cane lesions occur as a secondary stage of mosaic in the stems. No such condition is known in other plants which suffer from mosaic with the possible exception of potato in which "streak" seems to be closely related to mosaic. It is possible that "streak" may be a secondary symptom of potato mosaic although the necrosis is much more rapid and fatal than with sugar cane.

ROOTS

In spite of many careful observations no definite root symptoms are to be found other than a reduction in development or number of finer roots concomitant with the reduction in efficiency of the aerial part of the plants.

With sweet potato, however, Rosen (113) reports that growers lose as high as 60 per cent of their crop as a result of mosaic. Naturally a storage organ will more easily show the effect of reduced food supply, as in the case of potato tubers from diseased plants.

MODIFICATION OF SYMPTOMS BY TEMPERATURE

Conditions which affect the growth rate of plants may be expected to influence the symptoms of mosaic. In this connection Freiberg (58) observed that in tomato plants mosaic began to show from August 23rd to September 2nd during cool weather but when this period was succeeded by a relatively warm, drier period the symptoms began to disappear. There are two factors involved — temperature and moisture — but while both certainly have direct effect on the growth of the plant it is highly probable that temperature is the chief factor affecting the virus, as it is *in vitro*. Freiberg found quite different results with tobacco inasmuch as plants kept at 85° F. remained diseased while those maintained at 45° F. showed temporary recovery. In my experiments with tobacco I have found that mosaic symptons are not so obvious at low temperatures because leaf growth is slow and incubation time is lengthened. If the same plants be removed to a warmer house growth becomes more rapid and symptoms more pronounced. It cannot therefore be regarded as temporary recovery in such cases. In several instances I have kept tobacco plants, which were distinctly diseased at 70° F., for a period of 10 days or more at a temperature of 95° F. and in almost every case the symptoms were less pronounced in some of the older leaves and definitely so in the newly developed leaves, but on being returned to the temperature of 70° it was obvious that there was no evidence of partial recovery.*

Doolittle (45) notes the effect of temperature, especially soil temperature, on mosaic incubation in cucumbers and the resulting symptoms. At decreasing temperatures incubation time increased while at 30° C. new symptoms developed.

With potatoes I obtained similar results. Tubers from diseased stock were cut into two approximately equal parts and one lot of halves planted in a greenhouse maintained at 50° F. while the other lot was planted at 80° F. As the plants grew typical mottling with slight corrugation occurred at 60° F. but considerable curling with less pronounced mottling was found in all plants at 80° F. This is in accord with the view of Melhus (96) that curly dwarf is a modified form of mosaic due to different climatic environment.

In my tests with bean mosaic in the greenhouse I had considerable difficulty in obtaining good symptoms, although seed from the same lot gave 85 per cent severely diseased plants in the field, until I raised the temperature at which the beans were grown. The plants were still infectious in the 60-70° F. greenhouse but the symptoms were completely masked except in a few old leaves.

Similar conditions obtain with mosaic of *Pisum sativum* and of red, crimson, white and alsike clovers. With the clovers, however, there is apparently an optimum temperature for the "virus" somewhat below the usual maximum of a Quebec summer but I have not yet had time to determine it. *Melilotus alba* and *M. officinalis* likewise show most pronounced symptoms early in the summer and during the time of highest temperature symptoms are rather obscured. It is stated that sweet peas suffering from mosaic may recover (Taubenhaus, 137) and a similar condition to that described above may be involved in this case. In my experience I have not known a sweet pea plant with mosaic to recover.

I have not made any observations on the effect of moisture up to the present since I hold to the view that temperature is the more important factor, and in this connection only air temperatures have been considered although soil temperature of course affects plant growth.

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* Since the above was written Johnson has announced similar results (Abstract, Phytopath, 12: 52, 1922.)

AMOUNT OF INOCULUM

A further fact of interest in connection with modification of symptoms, and one on which more detailed work is being done by the author, is the correlation between amount of inoculum and growth condition of the plant in tobacco. I have found that when a seedling plant is heavily inoculated, as, for example, when three leaves out of five on the plant are rubbed with inoculum, the young leaves which have available a large amount of manufactured food, develop very marked distortion, savoying and mottling. The next leaf to develop is usually deformed and dwarfed but the savoying is less pronounced. The succeeding leaf is almost invariably dwarfed but not savoyed nor excessively mottled; in fact often the mottling is not at all pronounced. The same applies to the next leaf to develop if it can grow but such plants tend to remain *in statu quo* at this stage. It would appear that the infective principle, inoculated in such relatively large quantity, so affects the chlorophyll that photosynthesis is soon reduced so that a mere balance is maintained.

PATHOLOGICAL HISTOLOGY

In view of the present extensive list of described mosaic diseases it is highly important that an attempt should be made to correlate the histological and cytological symptoms of the plants affected. But little has been done in the past other than some work on tobacco, tomato and potato, and by comparing the results of Koning (77), Woods (144), Westerdijk (141), Heintzel (64) and Ivanowski (70) it is seen that they are not in entire agreement. Thus Koning found that chloroplasts were sometimes disorganized and intercellular cavities occurred between spongy and palisade parenchyma but in general believed that meagre results accrue from microscopic examination. Heintzel refers to the larger intercellular spaces of the dark green areas but does not discuss the palisade tissue of the lighter areas while Ivanowski especially mentions the larger intercellular spaces of the yellowed tissue and the underdeveloped palissade cells.

To A. F. Woods (144) is due the first finding of histological modification in leaves of tobacco plants suffering from mosaic although Koning had made microscopic studies with little result just prior to those of Woods. This author noted that in the lighter green areas palisade cells did not develop normally but were cuboidal or at most only about twice as long as wide as contrasted with four to six times in healthy cells. The passage from modified cells to ordinary cells was, as a rule, abrupt and the cells of the lighter areas contained more starch than normal at all times. He regarded the dark green cells as being healthy. These cells according to Ivanowski are such as would occur in a "vielleicht noch besser entwickelten Blattes, als desjenige einer gesunden Pflanze." Similarly Chapman (32) while using the term "healthy cells" says that "their structure may be somewhat modified by the increased functioning" thrown on them.

Two other mosaic diseases of Solanaceous plants have been studied histologically, viz. tomato and potato, and work has recently been reported on sugar cane (Matz, 86) and corn (Kunkel, 79). With tomato no characteristic abnormalities were found (Westerdijk, 141), or there was only a slight difference between lighter and darker green areas (Melchers, 94), while in the case of potato quite similar results to those on tobacco were obtained (Melchers).

In studying the pathological histology of diseased plants I have made major use of thin freehand sections of fresh tissues and always in contemporaneous comparison with similar tissues from healthy plants. The sections, even when mounted in water, were not used after it was thought that any changes due to treatment were occurring. Thus all measurements, descriptions and drawings are from freshly sectioned material. These have been carefully checked by the use of prepared slides from microtome sections but with our present methods of killing and fixing too much reliance cannot be placed on such preparations in the case of diseases of unknown etiology like mosaic.

I. TOBACCO. *(Nicotiana Tabacum)*

In a normal healthy leaf there is a clear differentiation between the palisade and spongy parenchyma. The palisade cells are from four to six times as long as wide, occupy two-fifths to one-half the leaf thickness, and are usually in one layer. The cells are well supplied with chloroplasts and this also applies to the spongy parenchyma. In both there are the usual intercellular spaces. Quite frequently there are to be found in the cells of the spongy parenchyma calcium oxalate crystals either of large size or in the form of "sand".

(1) LAMINA THICKNESS

In a diseased leaf, however, there is a striking difference in the lamina thickness of the light and dark areas, the relative thickness being 180-200 and 270-300 microns respectively, or a ratio of 2 to 3. This is referable mostly to the difference in development of the palisade tissue in the two cases as was shown first by Woods (144). In the light areas the palisade parenchyma cells do not lengthen or do so to a limited extent depending on the severity of the infection. In most cases when leaves are severely infected the cells are cuboidal or isodiametric (Pl. VI, Fig. 8) actual measurements showing variations from 20 to 60 microns in length and 17 to 28 microns in width. The cells of the spongy parenchyma in a healthy leaf are approximately twice as long as the palisade cells are wide, but in the light areas of diseased leaves the palisade cells are approximately equal in diameter to the underlying cells. This is due to the fact that the mesophyll cells are slightly smaller than in a healthy leaf, and that the normal division of cells in the palisade region is probably inhibited to some extent while the cells are meristematic. These cells are therefore in a hypoplastic condition as far as growth is concerned and the same applies to the cell contents as will be seen later.

By contrast the palisade cells of the dark areas are well-developed and in all cases they are at least normal in size. The writer has found, however, a very common modification when the disease is severe. Instead of the palisade cells occupying a single layer there are two layers, the lower comprising cells somewhat shorter than those in the upper layer but being obviously palisade cells. In such cases the individual cells are also narrower so that equal lengths of cross section from moderately and severely diseased leaves gave palisade cells in the proportion of 6 to 10 respectively counting the upper row only in the latter (Pl. VI, Fig. 9). This palisade tissue consequently occupies a relatively greater volume of the leaf and there is a considerable increase in the number of cells. Since they are well supplied with chloroplasts able to function we may assume that the photosynthetic efficiency of this tissue is increased. In view of the increase in volume it is obvious that the palisade tissue in severely diseased leaves is in a condition of hypertrophy and because of the increase in number of cells the hypertrophy may be accounted for by assuming a condition of hyperplasia when the tissue was meristematic. Woods (144) considered the palisade cells of the dark areas to be normal for he states that the "modified cells pass abruptly into the *normal* cells of the green areas". Chapman (32), however, while using the term "healthy cells" says that "their structure may be somewhat modified by the increased functioning thrown on the healthy cells". It is the writer's view that when infection is severe in any given meristematic cell that cell will be hypoplastic, but if it is infected later, or only slightly, then it will be stimulated either to hypertrophic growth or to hyperplasia. This would account for such instances above-mentioned.

(2) INTERCELLULAR SPACES

Spatial arrangement of the cells in diseased leaves varies in dark and light areas. It is the very regular arrangement of cuboidal palisade cells and respiratory parenchyma which allows light to pass easily through the yellow green areas. This fact was utilized in making photographs of diseased leaves by direct contact on a plate.

Mention has already been made concerning the differences of opinion relative to the histology and in order to determine what differences were present in diseased leaves use was made of squared paper and camera lucida drawings. Counts were made of the intercellular spaces in equal lengths of sections of the same leaf excluding the epidermis with results as below.

Relaties areas of intercellular spaces in transverse sections.

DARK GREEN			LIGHT GREEN	
	percentage of intercellular space in total mesophyll.	percentage of intercellular space in spongy mesophyll.	percentage of intercellular space in total mesophyll.	percentage of intercellular space in spongy mesophyll.
1.	21.3	33	13.6	22
2.	15.5	29	10.8	20
3.	14.6	27.5	9.8	17.6
Aver.	17.1	29.8	11.4	19.9

In 1, aver. of three old leaves.
In 2, aver. of three well-grown leaves.
In 3, aver. of three young leaves.

From the table it appears that there is an area of intercellular space approximately 50 per cent great in transverse sections of the dark green parts than in light green. Considering this on a volume basis the amount of intercellular space in dark green tissues is approximately three times as great as in light green tissues.

(3) TRICHOMES AND GLANDULAR HAIRS

Especially noticeable is the difference in the development of glandular hairs and trichomes on dark and light areas. There are considerably more on the yellow green than on dark green parts the disparity being greater than can be accounted for by assuming greater tension on the epidermis of hypertrophied parts and reduction in size of epidermal cells of hypoplastic areas. This feature holds good also in the case of diseased tomato, petunia and black henbane.

Many freehand sections have been studied and the following table gives four typical results for tobacco obtained by counting trichomes in a low power field.

Number of trichomes and glandular hairs.

Leaf	LIGHT		DARK	
	Upper	Lower	Upper	Lower
1.	7	15	3	7
2.	6	9	1	3
3.	6	7	0	1
4.	5	9	4	6
Total	24	40	8	17
Aver.	6	10	2	4

It is seen that there are 64 trichomes altogether on the light areas against 25 on the dark a ratio of 8 to 3 whereas by actual count the epidermal cells are as 7 to 6.

(4) CELL CONTENTS

Very marked differences were found in the cell contents and this was especially so in the case of the plastids. A normal palisade cell is well supplied with chloroplasts which line the periphery of the cell; starch grains in all stages of formation may be found; the nucleus is active and is usually placed midway the cell length.

But in the hypoplastic palisade cells of the light areas the number of chloroplasts which are apparently functioning is considerably reduced so much so that frequently there are not as many chloroplasts in these palisade cells as there are in the spongy parenchyma cells of the normal leaves. This lack of chloroplasts is the most striking characteristic of the contents of the severely diseased palisade cells. In a normal cell the chloroplasts when functioning under optimum conditions occupy a peripheral position in the cell but this is not the case with the chloroplasts in the cells under consideration for they are to be found very frequently irregularly arranged in the lower half of the cells. It is not unusual to see them accumulated on the lower wall or in the lower angle of the lower wall and a lateral wall (Pl. VI, Fig. 8.) Here also is to be found the nucleus in such cells, which may be considered barely top be actively living. The nucleus has not undergone any apparent change as a rule although I have observed occasional instances in severely diseased specimens where the nuclei were irregular in shape, being subspherical to angular, or considerably hypertrophied. Retrogressive changes in the chloroplasts are of frequent occurrence and appear to be of two kinds. In one they seem to be swollen and lighter in color than the remaining plastids and in the other they are broken down into minute, sometimes colourless grains mixed with the cytoplasm.

Severely diseased leaves were held inverted for five days and freehand sections made to determine whether, in cells having the cell contents congregated towards the lower wall, the contents had moved. In no case was any change in position observed, which fact indicates at all events a moribund, if not dead condition.

The chloroplasts not only are fewer numerically in the palisade cells of the light areas but, even if they are not breaking down as stated abbove and are normal in size, they are defintely yellower in color evidencing the presence of less chlorophyll in the plastids or of a changed chlorophyll. Moreover in many cases it appears as though the chloroplasts coalesce around the walls in the thin layer of protoplasm.

In those cells especially where the chloroplasts are congregated towards the lower wall of the cell and appear to be in various stages of degeneration, colourless, apparently crystalline, plates of various sizes and shapes are to be found. They are usually rather rectangular (Pl. V, Fig. 6) but occasionally rounded or trapezoidal in shape. While cells containing such plates are not uncommon in both palisade and spongy parenchyma of the light areas they are of very frequent occurrence in the trichomes and in guard cells of stomata of those areas. Guard cells having plate-like inclusions usually possessed no chloroplasts or but one or two lying close to the inclusion. Ewart (53) has shown that carbon dioxide combines with chlorophyll in the presence of water to form xanthophyll and a colourless waxy substance and that this is accelerated by bright sunlight. It is highly probable that these lamellae are of such a nature, that is, the product of chlorophyll degeneration combined with changed plastid protoplasm. When treated with 10 per cent hydrochloric acid the lamellae are etched and lined but not dissolved.

What has been said above applies also to the cells of the spongy parenchyma with the addition that crystals of calcium oxalate of varying size are to be seen in sections of healthy chlorenchyma but they occur more frequently and are of larger size in the cells of the light areas of diseased leaves. Occasionally too, pale waxy masses sometimes with a slight brown tint are to be seen in the cells of the yellow areas and it is likely that these are intermediate between coalescing chloroplasts and the hyaline lamellae above-mentioned.

Turning now to the dark areas the chloroplasts of the cells are to be found arranged parietally and they are in general more closely packed than in normal green leaves. Further they are somewhat darker in color thus giving these parts of the leaf their darker green color.

The narrower cells of the hypertrophic palisade tissue enable chloroplasts to be arranged parietally in more cells per given volume of palisade tissue and since they are as closely arranged as their functioning will allow there are more than the normal number of chloroplasts in such tissues. This fact points again to greater functioning by the darker green tissue and supports the view that there is a stimulative action in the dark areas.

The spongy parenchyma cells of the dark areas contain more and darker green chloroplasts. In no case has the writer observed the formation of the plant-like colourless structures seen in the cells of the light parts, and crystals of calcium oxalate seem to be a somewhat less frequent occurrence in the dark areas.

The stomatal guard cells in the dark parts possessed a full complement of chloroplasts and only in few cases were inclusions similar to those found in the stomatal guard cells of the light areas to be observed.

That the carbohydrate content is not the same in light and dark green areas is indicated by the iodine test. The chloroplasts and grains in the lighter green areas take on a brown stain, even when there is little obvious change in the chlorophyll apparatus, and the amount of carbohydrate is less because of the fewer chloroplasts.

The chlorotic condition of the lighter areas is not due to lack of iron in the plastid as is clearly indicated in the following test. Small pieces of tissue from lighter green areas of tobacco (and also potato, bean, pea and clover) were boiled in alcohol to remove fats and chlorophyll and then washed in doubly distilled water from Jena glass. They were then macerated slighty with clean glass needles on slides washed thoroughly so that no trace of iron, acid or alkali remained. Staining was carried out by using pure haematoxylin, when the plastids stained dark brown changed to deep blue thus indicating the presence of iron. Similar tests indicated that there was just as much iron present in the plastids of diseased leaves as in healthy. To prepare the stain, 0.3 grams of pure haematoxylin were washed with twice distilled water until the crystals were colourless. It was then dissolved in 50 cc. of doubly distilled water in a Jena flask and used fresh. (Macallum's method).

The only cells in which this was difficult to demonstrate for individual plastids were those containing many tiny hyaline masses resulting from their breaking down. It is possible, of course, that in such cases we have a hypoplastic condition under which the normal plastids did not develop originally but it appears more probable from my observations that this is a case of degeneration. A constant feature of such cells is the rapid movement of the small hyaline bodies. This movement does not appear to be entirely due to cyclosis and at present I cannot account for it.

Before leaving the discussion of the histology of the leaves it may be pointed out that once a plant is diseased all parts of the leaf blade must be considered as pathological, even though the tissues of the dark areas approximate in appearance those of healthy tissues. This is indicated by the fact that if dark parts are carefully cut out under sterile conditions and so that no trace of hypoplastic tissue is present, the juice from such areas is infectious to healthy plants. This fact has been consistently verified by the writer although in most cases the incubation period is of longer duration (14 to 20 days), indicating a less virulent infection in these cells.

STEMS. As far as the microscopic structure of the stems of diseased plants as compared with those of healthy plants is concerned the only difference so far determined is comparatively of little importance owing to the infrequency of its occurrence. It has already been mentioned that cases of badly diseased plants were observed in which on the upper part of the stem a quite definite streaking was found. The stem colour of a diseased plant is generally a very light green similar to that of the light areas of the leaves but in the cases in question there were very definite areas of dark green running down the stem in longitudinal strips as shown on Pl.VI, Fig.11. On holding the stem so that it could be examined tangentially it was seen that the surface of the dark strip was slightly raised above that of the surrounding light green tissues. Cross and radial sections showed that the chlorenchyma cells differed in the areas. In the dark green they were larger than in the light, there were more of them and they contained more chloroplasts, while in the dark green there were considerably fewer trichomes on the epidermis than in the light.

Cross and longitudinal sections of stems of all ages and stages of disease have so far evidenced no other change from the normal in structure. This is also stated to be the case by Chapman (32). The work of Quanjer (106) on Leaf Roll of Potato shows that there are phloem changes of a degenerative type which he denominates "phloem-necrosis" in that disease. Whether this is the cause of rolling of the leaves or a result is not yet settled but the phloem is certainly affected. It was thought that possibly similar results might be obtained in case of tobacco plants suffering from mosaic but no instances so far have been found.

The fact that no stem modification occurs except the ocasional one above mentioned and that no specific root modifications are observable, together with the fact that the only important histological changes of a pathological nature occur in the chlorenchyma, tend to show that mosaic in tobacco is a disease affecting primarily the chlorenchyma.

Roots. Roots and root tissues were carefully examined histologically but again no differences warranting any deductions were observed. If the plant is severely diseased the root system has a tendency to be poor but in all probability this is as much a result as a cause so that poor shoot and poor root are correlative.

II. TOMATO. *(Lycopersicon lycopersicon)*

Westerdijk (141) found no noteworthy modifications as a result of the disease in leaf sections except that in the yellow areas the chloroplasts are slightly smaller and paler than normal and develop but little starch and similarly Melchers (94) states:—"No striking differentiation was seen between the yellow and adjoining green or healthy tissues".

On the other hand the writer has found distinct differentiation in severe cases of disease such as were common in this vicinity during 1920 and 1921. Measurements of many sections gave an average thickness of 170 microns for dark areas as against 115 for light areas, a ratio approximately of 3 to 2 as with tobacco, etc. This is to be accounted for by retardation of growth in the palisade-cells which in many instances were actually cuboidal (Pl. VII, Fig. 23). In less severely diseased leaves the modifications were not so pronounced especially as the leaves matured, but in all cases there was a reduction in the length of the palisade cells. When they were cuboidal or nearly so they were slightly wider than normal being equal in width to the spongy parenchyma cells. This gives rise to a very regular arrangement of the cells in the light parts of the lamina as in tobacco, etc. (Pl. VII, Fig. 23.)

The upper epidermal cells of dark areas are longer than those of light areas but the latter are deeper actual measurements giving an average of 35-42 by 15-18 microns for the former and 24-35 by 15-24 for the latter. There is not so marked a difference in the lower epidermal cells.

More glandular trichomes occur on the upper epidermis of the yellow parts, the numerical ratio being frequently 6 to 1. The ordinary trichomes do not show such a marked difference but it is noticeable that more are to be found at the transition area between dark and light tissue. No difference was found in the case of the lower epidermis.

In severely diseased plants the transition area between light and dark parts is narrow as in tobacco, petunia, etc., but in less severely affected leaves there is a more gradual transition.

With the maturing of the leaves, especially during a dry spell, the light areas become very pale yellow and die prematurely.

The palisade tissue of the dark areas is well-developed and in the severely diseased plants the cells are somewhat narrower and greater in number than normal, indicating a condition of hypertrophy which is, however, not so pronounced nor of such common occurrence as in tobacco, henbane or petunia. The tomato appears to be more resistant to tissue change when infected by the mosaic disease "virus" than others of the Solanaceae under ordinary conditions.

As far as the cell contents are concerned similar, but less pronounced, characteristics obtain as in tobacco. The chloroplasts are fewer and paler, although there is little reduction in size, in the lighter areas. In the severe cases of the disease above-mentioned disorganization of the chloroplasts and cell contents is common and this results in the death of the cells of the light areas so that the leaves are flecked with dead brown tissue. No unusual formation of crystals or amorphous masses was observed.

Repeated histological examination of stem sections failed to reveal any necrotic phloem or any other marked structural modification resulting from the infection. Nor were any bacteria which could be used to infect healthy plants consistently found in the tissues.

The same facts apply to the roots of diseased plants.

As mentioned in the discussion of the symptoms there were many cases of fruit mottling. It was an easy matter to pick thirty or forty green tomatoes all showing very marked mottling on the upper half of each fruit from a few plants. The light areas passed suddenly into the dark and there was a distinct ridge rising to the dark green. Freehand sections showed that the chlorenchyma cells of the light parts are reduced in size and contained fewer chloroplasts. In the dark areas there were large chlorenchyma cells containing a maximum of chloroplasts. Immediately below the chlorenchyma the large cortical cells differed in their starch content, those below the dark green cells being full of starch while those below the light green contained little starch even during the hours of bright sunlight. This is obviously parallel with conditions in the leaves, the cells of the light parts are hypoplastic while the chlorenchyma of the dark areas is tending to hypertrophy. (Pl. VIII, Fig. 27).

III. PETUNIA. *(Petunia violacea)*

In diseased Petunia leaves the lamina varies in the thickness of dark green and yellow green parts the usual ratio being 3 to 2 but in many cases being as extreme as 2 to 1. Measurements of these thicknesses, dark green given first in each case, are as follows:—330 and 180, 390 and 260, 350 and 230 microns. This difference in thickness is due mainly to variation in the palisade tissue which in the dark green varied from 115 to 170 microns in depth and in light areas from being cuboidal to 80 microns. In severely diseased specimens all the palisade tissue is cuboidal or very nearly so (Pl. VII, Fig. 20) and such leaves frequently possess two rows of palisade cells in the dark green areas affording another instance of hypertrophy.

The cuboidal cells contain few chloroplasts, which are quite pale in colour and they are usually congregated in the lower half of the cell. In older leaves disintegration of the cell contents is of frequent occurrence and pale waxy masses in the form of plates are to be found in cells and trichomes. On the other hand cells in the dark green parts were abundantly supplied with fully-green plastids arranged parietally and functioning normally.

The epidermal cells over dark areas are larger than those over light parts but no marked difference occurs in the lower epidermis.

In both upper and lower, however, there were more trichomes and glandular hairs in the light green areas as compared with dark green. This fact accounts for the brighter appearance of the dark protuberances on the upper surface as in the case of tobacco, tomato and Hyoscyamus.

IV. POTATO. *(Solanum tuberosum)*

In typically diseased leaves the relative thickness of light and dark green parts is as 2 to 3, actual measurements varying from 90 to 100, and 120 to 150 microns respectively. The transition from thin to thicker tissue occupies a space of three or four palisade cells but in less severely diseased leaves the modification in thickness is less pronounced and the transition area longer.

The difference in thickness is due to hypoplasia of the palisade tissue in the yellow green areas and to the closer packing of the cells. The hypoplastic cells are either considerably shortened or entirely cuboidal and wider than normal (Pl. VIII, Fig. 30). The palisade cells of dark areas are approximately equal to those of healthy leaves, and in potato no definite case of hypertrophy has been observed.

The cells of yellow green parts are regular and closely packed thus reducing the volume of intercellular space as compared with that in healthy or in dark green tissue.

The chloroplasts of the cells of the light areas are fewer in number and paler in colour than in the dark green but there are fewer instances of chloroplast degeneration than in tobacco. In the dark green tissues the chlorophyll content is always at least equal to normal and sometimes above that of healthy tissue.

Calcium oxalate crystals in masses of a size equalling in width two palisade cells are of common occurrence in the dark green tissue but less frequent in the light.

Slight differences are observable in the size of upper epidermal cells in the two cases those of the light areas being smaller and an increase in the number of trichomes in yellow green parts is sometimes quite pronounced. This is not referable to the smaller size of those epidermal cells but is an actual increase in number.

Sections of leaves from plants showing the dark green curly type of mosaic were made but no cases of hypoplasia were found. Where the lamina was flat the cells seemed to be normal there being little difference in cell size or cell contents. In the curled, bulging areas the cells were closely packed and filled with dark green chloroplasts as though again there was a tendency to hypertrophy. Only in a few instances was any unusual movement in the cells noticeable and it would appear that under higher temperature conditions mosaic symptoms are modified as has previously been suggested in discussing symptoms.

No stem modifications in ordinary mosaic have been found nor are any unusual histological changes to be observed in the tubers. In order to see if mottling of the tubers might occur plants were grown in the greenhouse and when tuber formation was about to begin either the stolons were kept in daylight or were cut off entirely. In the former case small dark green tubers developed, and in the latter aerial tubers grew in axillary positions. In neither case was any mottling or other modification visible.

STREAK AND LEAF DROP

Güssow (62) found in severely diseased potatoes a necrotic condition in the leaves of several plants. The necrotic areas were not large and occasionally dark streaks show in the peticles. Orton (102) discusses a similar condition which he observed from 1912. The "streak" is characterized by elongated or angular spots following the veinlets and invading the parenchyma. Shortly after these spots appear the petiole collapses and the leaf withers and falls or hangs by a thread from the stem. Such leaves when first affected are brittle, the upper leaves being affected first and gradually the stem shows long, faint brown streaks, eventually dying.

The writer, in working with potato mosaic in the greenhouses found that certain Green Mountain plants affected with mosaic developed "streak" or "leaf drop" in many instances. This was so much the case that out of twelve plants grown in February of 1920 not one could be used for ordinary mosaic studies owing to the severity of the "streak." No similar symptoms appeared in any of the healthy check plants. The plants grew rapidly and mosaic infection exhibited itself very early and was of the advanced type. When they were approximately fifteen inches in height slight necrotic lesions appeared near some of the smaller veins of the uppermost well-developed leaves of eight plants. At the same time there were one or two streaks of brown tissue, slightly sunken, and about a quarter of an inch in length, in the petioles of each of these leaves. The lesions increased rapidly in the tissues of the lamina and within two days the leaves wilted, the leaf blades dried out and the petioles withered from the midrib back towards the stem. In addition the petioles separated from the stem altogether or remained hanging by a strand of epidermal tissue on the lower side. Similar lesions appeared in the stems at this time. On the other hand in four of the plants the leaves dropped from below upwards so that eventually there was a bare stem surmounted by a tuft of leaves at the top. But while the uppermost leaves did not exhibit similar symptoms the flowers did and these were marked with necrotic brown streaks beginning usually near the middle of each corolla lobe and gradually increasing in number until the lobe was plentifully streaked. By this time the flower falls since the pedicel is affected similarly to the petioles of leaves but if by chance it remains attached necrotic lesions begin to appear in the anthers of the stamens.

This condition cannot, in the light of present knowledge, be definitely ascribed to mosaic but it certainly appeared in plants known to be affected with mosaic and not in non-mosaic plants. Moreover tubers from such plants, grown in the field or greenhouse, again showed both mosaic and streak when the plants were well developed. In early stages of lesion development in stem and petiole browning is to be observed in the phloem elements and in cortical cells but not in epidermal cells. Later brown granular deposits are seen in xylem elements; the phloem parenchyma and some phloem groups may be quite necrotic and groups of cells in the cortex show distinct signs of breaking down. In advanced stages necrosis occurs from the vascular elements out to the epidermis. The small veins in the lamina, however, were necrotic in the tracheids (Pl. VIII, Fig. 31) and more rarely in phloem and parenchyma at first. The lumen of a necrotic tracheid was found to be filled with a brown granular deposit and the walls took on a deep stain with Magdala red while healthy tracheids were unstained. Later both palisade and spongy parenchyma cells were affected, the former to a greater extent. Frequently one or two palisade cells were necrotic and isolated, even the epidermal cell above them being apparently unaffected. If the spongy mesophyll cells below were disorganized the lesion took in the whole thickness of the lamina including both upper and lower epidermis.

In the palisade tissue it seemed as though the cells were attacked from the intercellular spaces for the first signs of necrosis appear in the parts of the cell walls bordering the spaces. Very frequently these small spaces are filled with a dark-staining mass of disorganised material. Gradually the whole wall is involved and the cell contents begin to die. The latter form a disorganised granular mass in the cell except for the nucleus which usually retains its shape but becomes deep brown in color (Pl. VIII, Fig. 31). When the necrotic tissues take in the entire thickness of the lamina the cells collapse and shrink giving rise to the dead areas so noticeable in the leaf.

My observations show that in all the sections studied no lesions were found in the darker green bulging parts of the leaf but they all occurred in areas which were less developed although they were not definitely hypoplastic.

V. BLACK HENBANE. *(Hyoscyamus niger)*

In *Hyoscyamus niger*, as with other mosaic diseases, very marked difference in leaf thickness occurs, that in the ligth parts being only half to two thirds that of the dark (Pl. VII, Figs. 17, 18).

The difference in thickness is due to hypoplasia of the palisade tissue in light areas and in severe cases also to hypertrophy of the palisade tissue of dark areas (Pl. VII, Fig. 17), this latter condition being common in *Hyoscyamus.*

Usually the palisade tissue in yellowish areas is entirely lacking the cells being cuboidal. The cell contents differ as in the case of tobacco the differences being so similar that further mention need not be made of them here.

Spatial arrangement of the cells in the dark and light areas is also comparable with that in tobacco, tomato, raspberry, etc., suffering from mosaic and, as in tobacco, petunia, etc., there are more trichomes on the epidermis of the hypoplastic areas than on healthy and dark green tissues.

The relative numbers of trichomes and glandular hairs is shown in the following table, counts being taken in equal lengths with a low power field.

This gives a proportion on the average of nearly 4 to 1 for upper surfaces and 5 to 2 for lower, or 3 to 1 for both, whereas the relative sizes of epidermal cells is 3 to 4.

Therefore again there is definitely an increase in the number of trichomes irrespective of any modification of epidermis in the yellowed areas.

	YELLOWED AREA.		DARK AREA.	
Leaf	Upper	Lower	Upper	Lower
1	10	13	2	3
2	11	15	3	4
3	10	8	2	5
4	10	12	4	4
5	18	13	5	8
6	6	8	1	3
Totals	65	69	17	27
Aver.	10.8	11.5	3	4.5

VI. PEPPER. (*Capsicum annum*)

No modifications of stem or root structures were observed in the case of pepper as in other mosaic diseases and the leaves exhibited the usual typical changes.

The lamina in yellow green areas was thinner than in dark green parts measurements giving 210 to 235 microns and 275 to 290 microns respectively. This proportion is approximately 4 to 5 as against 2 to 3 for most of the plants studied.

The difference in thickness was due, as in other cases, to hypoplasia of the palisade tissue together with a closer arrangement of the spongy mesophyll cells in lighter areas, (Pl. VIII, Fig. 26). No instances were found in *Capsicum* where cuboidal palisade cells were present, yet the difference in palisade tissues was marked and the transition from hypoplastic to dark tissue was abrupt in those cases where larger yellowed areas obtained.

Parallel conditions are found in the chlorophyll content of the cells. In the darker areas cells contain a full complement of chloroplasts all full green in color whereas in the yellowed areas there are fewer chloroplasts and these possess less chlorophyll.

In severely diseased leaves the chloroplasts in both palisade and spongy mesophyll of lighter areas have a tendency to congregate in the lower part of the cell and frequently they are to be found there altogether or partially disorganized (Pl. VIII, Fig. 26).

Inclusions, of varying size, of calcium oxalate are to be found in both dark and light areas but with greater frequency in the dark. This is similar to the result found in potato mosaic, and different from that in tobacco.

VII. SWEET PEA. *(Lathyrus odoratus)*

In diseased leaves the leaf thickness varies, that of light areas being 108 to 120 and in dark parts 160 to 175, and again the difference in lamina thickness is due to hypoplasia of the palisade tissue the cells of which in light parts are cuboidal (Pl. VII, Fig. 22) and in dark 60 by 12-15 microns. The transition from palisade to cuboidal cells is sudden thus sharply delimiting the areas.

No case of hypertrophy was found in the plants studied except in so far as the dark green chloroplasts are concerned and these are somewhat more numerous than normal.

In most of the severely diseased leaves the chlorophyll content of the cells in the light areas was reduced and the chloroplasts were disintegrated so that the lower parts of the cells contained a green granular mass with no individual chloroplasts (Pl. VII, Fig. 22).

The chlorenchyma cells in close proximity to those with disorganized plastids frequently contained small hyaline bodies which were in rapid oscillatory motion, quite similar to the condition in tobacco, etc.

Sections through mottled corolla parts in addition to showing the sudden change from coloured to colourless cellsap at the same time proved that here too the white tissues were hypoplastic.

VIII. KIDNEY BEAN. *(Phaseolus vulgaris)*

The leaf symptoms in the case of kidney beans may be fairly pronounced but, exactly as with the Solanaceae, it is not always possible to find areas showing sudden and clearly marked transition from hypoplastic to non-hypoplastic tissues. Conditions of growth modify the influence of the "virus" on the tissues but in rapidly grown diseased leaves conditions comparable with those in diseased tobacco are to be found.

Thus in young diseased kidney bean leaves the lamina thickness is 110-180 microns in yellow green parts as compared with 170-280 microns in dark green tissues. This is referable again to the hypoplastic condition of the palisade tissue in lighter areas combined with some hypoplasia of the spongy mesophyll cells and a very regular arrangement of all the cells. In some instances the hypoplastic palisade cells were 52 microns long by 17 to 25 wide while in others they were 25 microns long by 20 to 25 wide. There are all gradations from nearly normal to cuboidal cells. Similarly in the spongy mesophyll cells in the dark green are commonly 25 microns wide by 50 long whereas in the lighter green they are 17 to 20 by 35 microns.

When leaf growth has been particularly rapid hypertrophy of the palisade tissue may occur resulting in two rows of cells, totalling about 135 microns of which the lower cells are somewhat shorter than the upper. I have not noticed any tendency to narrower cells in hypertrophied tissues of bean such as occurs in tobacco, petunia, hyoscyamus and raspberry.

Cells of lighter areas are deficient in chloroplasts and those present are paler than normal. Instances are frequent where the plastids appear to have broken down into minute bodies which become hyaline and are in a state of rapid oscillatory motion. Again there may be a congregating and coalescence of plastids in irregular groups or masses in the lower parts of cells. The conditions are so similar to those occurring in tobacco that no more details are needed to prove the histological similarity of the two diseases.

Still better material is to be found in a climbing Kidney bean known as Cluster Wax. Cuboidal cells in the place of palisade cells are common throughout comparatively large areas of tissue and hypertrophy is also pronounced in small areas. Accompanving the cuboidal cells of the palisade is the reduction in size of the spongy mesophyll, the very regular cell arrangement with a minimum of intercellular spaces and the other cell features concomitant with these as described in tobacco mosaic.

Trichomes similarly vary in number over lighter and darker areas as is shown in the accompanying table. Equal lengths of representative tissues were observed in low power fields.

Number of Trichomes

LIGHTER		DARKER	
Upper	Lower	Upper	Lower
7	10	3	4
4	11	4	5
7	8	3	5
Total 18	29	10	14
Aver. 6	9 2/3	3 1/3	4 2/3

There is an approximate proportion of 2 to 1 for light and dark green parts respectively, and this cannot be accounted for by supposing a proportionate epidermal cell size since in that there is little difference observable.

No constant histological abnormalities have been found in stems, roots, flowers or fruit in spite of diligent search.

IX WINDSOR OR BROAD BEAN

(Vicia faba)

The darker tissues are closely packed with chloroplasts whereas there are considerably fewer and paler chloroplasts in hypoplastic cells. In some cases disintegration of the chloroplasts was observed in cells of hypoplastic areas. In general the microscopic characters are so similar as not to warrant repetition except to note the fact that in *Vicia faba* the cell arrangement is looser giving considerably more intercellular volume than in ordinary beans.

Comparison of light and dark areas gives similar results as in the other mosaic diseases. The dark areas are one and a half to twice as thick as light parts and this difference is due to modification of the palisade tissue in the paler areas. Here the palisade cells are either cuboidal or at the most but slightly elongated (Pl. VI, Fig. 13) whereas in the adjoining dark tissue the cells are four to six times as long as wide (Pl. VI, Fig. 12).

X. CLOVERS

In a later part of the paper I have discussed infection experments with various clovers some of which are noted for the first time as being susceptible to mosaic. They do not show symptoms equally well, however, and in placing them in descending order according to symptom prominence we have crimson, red, alsike, trefoil and white clovers.

The gross symptoms are paralleled by the histological and thus it is in crimson and red clovers that isodiametric palisade cells are common while a less pronounced hypoplasia is the rule with black medick, white Dutch, and alsike clovers. Nevertheless the facts are entirely in keeping with what we know of other mosaic diseases. Leaf thickness in red clover varies from 105 microns in the lighter to 150-180 in the darker and where it reaches 180 microns there is some tendency to hypertrophy, while with crimson clover the lamina thicknesses are 150 for light and up to 250 microns for dark green parts. Chloroplasts are fewer and paler in lighter areas and the intercellular spaces are reduced somewhat by the muriform cell arrangement. In crimson and red clovers the contents of severely infected cells tend to disorganize and when this takes the form of the breaking down of chloroplasts into small hyaline structures there is evidenced again the same excitation which has been noted with tobacco, tomato, etc.

XI. CANADA FIELD PEA. *(Pisum sativum)*

This is a new host for mosaic but the symptoms and histology are so like those in Kidney beans and sweet pea that it is not necessary further to mention them beyond the fact that they are often not pronounced.

XII. RASPBERRY

Raspberry mosaic is dealt with in detail, except for the histology, in another part of the paper owing to the fact that it has not previously been recorded specifically as a mosaic disease (Dickson 42).

Examination of leaf sections serves to uphold this view for dark green, bulging areas have a lamina thickness of from 90-105 microns while in lighter areas it is only about 62-70 microns. In these thinner parts hypoplasia is marked, palisade cells are cuboidal, spongy mesophyll cells smaller and closely and regularly arranged while in the darker parts either palisade cells are normal in size or this tissue is hypertrophied. Chloroplasts are fewer in the cells of lighter parts and, depending on the severity of infection and the season, there are all gradations of disorganization and degeneration. If the weather be very dry flecks of brown tissue begin to appear in the light areas due to death of the cells. A noticeable feature of the hypertrophied tissues is the presence of large cystoliths in a space equal in volume to two mature palisade cells. These rarely occur in hypoplastic tissues and when they do are necessarily smaller.

The epidermal cells overlying the hypoplastic areas are slightly smaller in area but somewhat deeper than those over the normal tissues and, in cases where this increase in depth does not occur, there are more trichomes on the lighter green parts than on the bulging, dark green areas.

There is a complete parallel between the microscopic symptoms in the case of raspberry mosaic and other mosaic diseases.

SUMMARY OF THE RESULTS OF MICROSCOPIC STUDIES

Previous studies by other authors have been concerned chiefly with tobacco and in one or two cases with tomato and potato. In these no agreement is reached except in the finding of hypoplastic palisade tissue in lighter green areas of tobacco and potato and of fewer and paler chloroplasts in those areas.

As a result of my studies with tobacco, tomato, potato, black henbane, petunia, pepper, sweet pea, Canada field pea, kidney bean, Windsor bean, raspberry, red clover, crimson clover, alsike clover, white Dutch clover and black medick it is clear that certain microscopic features are common to all the mosaic diseases studied. It is not to be understood, however, that in any leaf section from a diseased plant all characteristics, equally well developed, are to be observed. Much depends upon the growth rate and environment of the host plant but if sections are made from rapidly grown leaves of well-infected plants most of the following findings can easily be verified.

There is a difference in the lamina thickness of light and dark parts of the leaf varying in amount and in the majority of cases giving a relative value of 2 to 3 respectively where the disease is ordinarily severe. If the infection be slight there is less reduction in thickness of lighter green parts but if the attack be unusually severe there is more reduction in the lighter parts and an increase in thickness of such parts as are dark green. Usually the dark green is but little thicker than an ordinary healthy leaf. This reduction in leaf thickness is due to hypoplasia of both palisade and spongy mesophyll cells but more especially to the former. There are all gradations from slight hypoplasia to that in which the cells are isodiametric and can only be determined as palisade cells by their position. It would appear that the hypoplastic condition of such cells is indicated also by the fact that they are usually somewhat wider than normal palisade cells. In a healthy leaf of tobacco for example a spongy mesophyll cell subtends approximately two palisade cells. Allowing that there is hypoplasia of the spongy mesophyll cells their reduced size is not very marked and yet only one hypoplastic palisade cell is needed to equal one mesophyll cell. This will be referred to again but the point of view with regard to these palisade cells is, that not only are they unable to develop in length but they must have been inhibited from further division when meristematic. In leaves where the attack is mild the palisade cells are narrower and longer, in fact nearly normal.

The spongy mesophyll cells are also hypoplastic to a greater or less extent and when the disease is more severe they are equal in length to the width of the palisade cells above them. There is thus a very noticeable regular arrangement of the cells in the lighter green leaf parts. This markedly muriform arrangement allows more easily the transmission of light as there is considerably less reflection from walls at an angle. This fact has been taken advantage of in making the photographs in the plates as all with one exception were taken by direct contact on the photographic plate.

This regularity in cell arrangement necessarily reduces the proportion of intercellular space in the leaf, and by contrast there is usually an increase over normal in the intercellular spaces of hypertrophied parts.

The epidermis over hypoplastic areas is composed of cells somewhat smaller in area than those over normal tissues. If they are smaller superficially, however, they are usually deeper than normal. This is quite marked in raspberry, and sometimes in clovers and sweet pea. It is probable that this greater depth is a result of the fact that growth in area is limited by the hypoplasia below and the volume growth is therefore made up by an increased depth to the cells. Of a similar nature is undoubtedly the increase in number of trichomes on lighter areas of severely diseased leaves so marked in tobacco, henbane, etc. It is probable that if tangential cell growth is impossible because of the excessive hypoplasia below, division and growth into trichomes occur.

This is in a measure borne out by the fact that there are definitely fewer trichomes than normal over such dark green bulging parts as are in a condition of hypertrophy. Here tangential epidermal cell growth could proceed to a maximum.

Corresponding with the hypoplastic condition of the cells there is a gradation of hypoplasia and degeneration of the cell contents. Cells, slightly affected as far as growth is concerned, may be different from healthy cells only in the slightly paler green of the chlorophyll while the choroplasts appear to be present in nearly normal number. With increase in the severity of effect on cell size there is a very definite reduction in the number and depth of colour of the chloroplasts and in some instances they are also reduced in size.

Still more acute infection results in the disintegration of the chloroplasts or in their coalescence into irregular green masses in the lower parts of the cells. When disintegration occurs the chloroplasts lose all their green colour and break up into many small hyaline granules of varying size and in cells containing such bodies a marked feature is the excessively rapid movement of the contents. The rapidity of the movements of the granules is greater than can be accounted for by ordinary protoplasmic streaming. Very often among the oscillating bodies smaller ones may be seen under high magnification exhibiting irregular rapid movements. On staining with I in KI the larger bodies take on a brownish coloration but the smaller ones are not affected. No satisfactory reason can at present be given for this condition.

In other cells of the same leaf section a typical agglomeration of the chloroplasts may occur. They appear to be drawn in the protoplasm toward the deeper halves of these cells. The chloroplasts may be grouped in one large mass or in two or three smaller masses. Coalescence of the plastids occurs resulting in an irregular, uniformly pale green body in which the nucleus may be buried altogether or partly.

Gradually the chlorophyll disappears and the masses become more or less hyaline or waxy and slightly brownish. It is from these that the writer believes the plate-like, waxy, semicrystalline bodies common in palisade and spongy cells and in trichomes arise.

Similar effects to those just mentioned are to be found in even moderately infected leaves when they have been living for a considerable time under such conditions.

This extreme effect over large numbers of cells in the hypoplastic tissues gives rise to the dead flecks in the leaves seen when the plants are subjected to rather sudden adverse conditions or to more prolonged but less severe drought.

Among the Solanaceae, especially in tobacco, petunia and henbane, the small hyaline bodies and the waxy-crystalline lamellae are very common in the trichomes. When the small bodies are seen they are always in rapid motion and when the plate-like structures are found the amount of protoplasm appears to be reduced leaving a larger central vacuole than usual.

Referring now to the dark green areas of the leaves there is a similar gradation in depth of dark green and in the extent of hypertrophy. There is certainly a greater volume of intercellular space in darker tissues and this tends to make those tissues appear darker because of the reduced amount of light passing through them. Nevertheless there is, in dark green bulging, savoyed parts, a definite hypertrophy. The cells are larger than normal, contain more chloroplasts and the chlorophyll is slightly darker green than in a healthy leaf. When the savoying is very marked the palisade cells are in a condition of hyperplasia. Either they have divided to form two layers of palisade cells or they are narrower, longer and more numerous or both may have occurred. This condition is common in tobacco and henbane, frequent with petunia, raspberry and beans (Cluster Wax) and has been found in severely affected tomato, red clover and crimson clover.

Where hypertrophy occurs the breaking down or coalescence of chloroplastids has not been observed.

No stem lesions or modification of stem tissues have been found with the exception of the dark striping on tobacco and in this case the affected tissues were chlorenchymatous. Nothing comparable with phloem-necrosis such as is observed in Leafroll of Potato occurs and in fact vascular tissues are apparently unaffected.

In contrast with this it is interesting to note that with corn and sugar cane (Matz, Kunkel) internal cankers due to necrosis of the parenchyma and later of vascular tissues are common in severely affected stalks. This internal lesion is quite like that occurring in potato suffering from "streak". A further difference between corn mosaic and those studied by the writer is that lighter green areas are thicker than dark green owing to the hypertrophy of the cells (Kunkel) whereas exactly the reverse is the case in all the mosaic diseases described in this publication.

Root tissues are not affected and the only difference between roots of normal and diseased plants is that the latter are usually less developed correlative with poorer shoot development.

Tissues of floral organs do not as a rule exhibit histological modifications but in tobacco, sweet pea and petunia the groups of cells without colored pigment are in a condition of hypoplasia, more or less marked. Fruits, likewise, when they do present tissue modifications (tomato often, pepper rarely) are affected only in the chlorenchyma the conditions being quite similar to those in the leaf.

In conclusion it is obvious that in the mosaic diseases studied the tissues directly affected are chlorenchymatous. Indirectly the epidermal tissues are modified and because the chlorophyll apparatus is reduced in efficiency the plants are dwarfed in correlation with the severity and duration of infection.

ETIOLOGY

The mosaic group of diseases is intriguing because of the unknown etiology of each single member of the group and herein lies a danger that an over-anxiety to determine the etiology will cause one to assume too readily that the presence of organisms, their successful culture and apparently successful inoculation into a particular plant has solved the problem. It is always possible that the causal principle has been carried along in the cultures with the other organisms as entirely secondary features.

Nevertheless the fact that in human medicine similar problems are gradually being solved leads one to hope that the solution of the etiology in mosaic diseases is not to be long delayed.

Several theories have been advanced each with some foundation in fact even though the facts do not warrant all that is stated of them.

1. Bacterial Theory

Naturally one which is prominent is the bacterial theory promulgated by Mayer (87), Ivanowski (70) and Boncquet (24) among others. The work of Ivanowski is to my mind the most convincing inasmuch as I have found structures similar to those he found, in tobacco and also in henbane, potato and bean. He found four types of structure in the palisade cells of yellow areas of diseased leaves, viz:—bacteria, amoeba-like bodies, colourless lamellae and deposits of a waxy-crystalline nature. As to the bacteria, which may form zoogloeæ, they are very small rod-like organisms 0.3 microns long: Ivanowski figures them and also gives photomicrographs. They are found near the cell walls rather than in the cell sap. In fresh cultures they form long threads and with gelatin the medium is stained dark and sometimes liquefied. He states that infection was successful but apparently not enough was done actually to prove this beyond doubt.

Hunger (66) too found minute, bacteria-like bodies and occasionally plasmodium-like structures. While studying Curly top of sugar beet, Boncquet (26) described an organism *(B. morulans)*, inhabiting the sieve tubes of diseased plants, which was an active nitrate reducer. This author in a short paragraph (24) states that in tobacco he found an exceedingly small streptococcus which was also vigorous in reducing power. It is rather to be expected that whatever does cause the disease will be a nitrate reducer inasmuch as the chlorotic condition of the tissues is not due to lack of iron in the plastids according to my tests, above discussed.

In the present state of our knowledge the bacterial theory, while not entirely discarded, cannot be considered as other than a working theory, nor has it gained any general acceptance.

2. Enzymatic Theory

Another group of investigators, Woods (142), Heintzel (64), Chapman (30), Freiberg (58), etc., have advanced the enzymatic theory. Heintzel concluded in his account of the disease that the enzyme which causes the mosaic disease of tobacco is known as an oxidase and Woods states that "it seems plausible that in rapid, poorly nourished growth many of the cells were unable to develop their normal amount of chlorophyll by reason of the excessive development of oxidising enzymes". The excessive oxidase development inhibited diastatic activity in the cells and thus accounted for starch accumulation in diseased cells. It is pertinent here to remark that in confirmation of the results of Freiberg (58) I have not found that there is any appreciable starch accumulation in the yellow-green areas of diseased leaves, and in fact there is generally more carbohydrates in the dark green than in lighter green areas.

So far as oxidases are concerned Allard's experiments are interesting. He destroyed oxidase by hydrogen peroxide but still obtained an infective principle, while by destroying the "virus" and leaving the oxidase no infection was obtained. It of course does not preclude the possibility of an aldehydase being the causal principle as stated by Freiberg (p. 217) but his statement that "from the above it is obvious that when injecting the infected substance obtained from a diseased plant into a healthy plant, we are handling an enzyme and not an organism" is not warranted as yet.

3. VIRUS THEORY

Beijerinck (13) although he first favoured the bacterial theory, proposed in its place the theory that mosaic disease is caused by a "contagium vivum fluidum". According to this theory, the causal principle is non-corpuscular and water soluble. His view that it is a liquid is due to the fact that he found it diffusible through agar but with our present knowledge of ultrafiltration this evidence is open to criticism. Lodewijks postulates not only a virus but also the development in healthy tissues of an antivirus (See p. 59.).

Regarding the term "virus" in a general sense to include an ultramicroscopic organism or an infective principle of unknown type a recent paper given by Duggar (51) is of great interest. Using ultrafilters, of which the pore diameter could be determined, he found that the particles of the infective principle were in the vicinity of 30 to 40 millimicrons. This is of the highest importance and it is to be hoped that a detailed account of the work will soon be published.

4. "AMOEBA" THEORY

Lately two investigators (Matz, 86, and Kunkel, 79) have published results of studies in which they suggest the possibility of an amoeba-like body being the cause of mosaic in sugar-cane and corn. Matz found in parenchyma cells of internal canker of the stem of sugar cane, and in leaf sheaths, a peculiar densely but finely granulated and slightly browned plasma. Usually there are a few cells so filled in a group. Dotted throughout the mass are hyaline bodies less than one micron in length and in a test of externally sterilized material kept in a moist chamber motility was observed ofter eight days. This substance "resembling a Plasmodium" was found to be "constantly associated with yellow-striped cane in an advanced stage of the disease". Kunkel (79) corroborates the findings of Matz and furthermore states that similar "plasma"-filled cells are observable in diseased sugar cane tissues. But he describes an intracellular body of a different type. These show great variation in size and in early stages of the disease are so minute that it is difficult to find them under high magnification. Finally they reach a size where they are conspicuous. They are never spherical but are amoeboid and always closely associated with the cell nucleus, in fact usually attached to it. The host nucleus enlarges and is often deeply imbedded in the body. Sometimes a thin membrane is present but in most cases the body appears to be naked and all attempts to demonstrate a nucleus have failed. Host cells containing these bodies may enlarge rapidly or rather slowly and it is usual to find that the more rapidly hypertrophied cells break down the more readily.

In connection with this it is interesting to recall that Ivanowski described and depicted somewhat similar bodies closely appressed to the nuclei in tobacco mosaic.[1]

4. "VIRUS" DISEASES OF ANIMALS AND MAN

The fact that in most mosaic diseases so far described insects, as possible alternate hosts, play a part inevitably leads one to consider similar diseases in human and animal pathology. Space conditions prohibit a detailed account but mention may be made of a few illustrative cases. The term "filterable virus" is the designation applied to the infectious principle in which filtration through a Berkefeld filter does not present infection when the filtrate is introduced via the proper atrium by the agent of infection. In affected cells are usually found cell inclusions or chlamydozoa which are minute bodies less than 1 micron in size supposed to be protozoan in nature and consisting of a minute "elementary body" enclosed by a mantle from the reacting host cell. Some do not become cell inclusions but remain as elementary bodies — "chlamydozoa strongylo-plasmata". Loefler and Frosch in 1898 proved that "Foot and Mouth" disease was caused by a filterable virus, this being the first instance in animal and human diseases. It will pass through the coarser filters and is killed in 10 mins. at 50° C.

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[1] *Mycoplasm theory.*—Eriksson has suggested the applicability of his mycoplasm theory to tobacco mosaic and, while it is not suggested here that mosaic is caused by a "myco"-plasm, it may be that in mosaic diseases there is the possibility of the nearest appraoch to such a theory. (Phytopathology 11: 388, 1921).

The virus of rabies will pass through a Berkefeld filter and is killed at 50° C. In some cases amoeboid forms 18-23 microns are found but with "fixed" virus in nerve tissues the bodies are 0.5 microns or less.

In measles loss of infectivity of the virus occurs after 15 mins. at 55° C. and in the case of Poliomyelitis globoid bodies 0.15-0.3 microns have been found by Flexner and Noguchi.

Kruse in 1914 argued that coryza (common cold) is due to a filterable virus and in tests nasal discharge, diluted 10 to 15 times by saline, filtered through a Berefeld, produced the disease. The filtrate in the cases of fowl pest and pleuropneumonia of cattle through Chamberland filters is killed at 58° in 30 minutes. It has now been demonstrated that the organism causing pleuropneumonia of cattle is a pleomorphic coccoid body visible at 1500 magnification.

5. PRESENT STUDIES CONCERNING ETIOLOGY

During the course of these investigations I have inevitably been concerned with a possible solution of the problem presented by the etiology of mosaic and the following statements constitute a progress report.

In no instance among the many hundreds of sections studied have I observed any bodies such as those described by Kunkel in corn mosaic. There are occasionally to be found more or less amoeboid hyaline bodies in diseased cells of leaves but in such cases it would appear more probable that they are secondary in nature.

On the other hand, in freehand sections of leaves in advanced stages of the disease there are often to be seen, among the hyaline bodies in spongy mesophyll cells and trichomes, smaller bodies having an erratic movement. They appear, so far as can at present be ascertained, to be like a flagellate but in spite of careful killing no definite proof of this can be drawn from non-living sections. A bacterial flora is almost invariably present in diseased leaves and in cultures made it has been impossible to isolate a protozoan organism from the cultures. This may be due to a change in the form of the organism if indeed a protozoan be present.

In sections of tobacco killed in a concentrated alcoholic solution of mercuric chloride and stained according to the Giemsa method minute dark staining bodies 0.3 microns long and slightly less in width are to be seen, sometimes in great number, in the border parenchyma of the vascular tissues of diseased leaves and these do not occur, so far as my observations show, in healthy leaves. They may also be observed in close contact with the walls of the chlorenchyma cells and in some cases surrounding the chloroplasts. These are apparently the same bodies observed and pictured by Ivanowski (70) and possibly the same as those mentioned by Boncquet (24) and Hunger (66).

The fact that they do occur in diseased tissues does not prove that they are other than secondary or symbiotic but it is nevertheless possible that they are akin to the chlamydozoa mentioned in the discussion of "virus" diseases of animals and man.

I have made many attempts to culture them and have obtained infection from such cultures. The method used is, however, open to criticism; nevertheless it may not be valueless to describe it. Small pieces of hypoplastic tissue were cut out and washed for 2 mins. in absolute alcohol and transferred to mercuric chloride solution (1 in 500) for 3 mins. They were then washed for 10 mins. in three changes of sterile water and transferred under sterile conditions to bouillon tubes. In placing them in the sterile bouillon they were slightly crushed with sterile forceps. A layer of sterile melted paraffin was poured over each culture which was thus sealed. The tissues gradually discolored and in two days at room temperature a slight turbidity appeared. Examination of the cultures showed the presence of minute organisms, sometimes in zoogloeae and sometimes in streptococcus-like chains. From such cultures inoculations were made using 1/2 cc. per plant on a total of 15 plants. In twelve out of the fifteen typical mosaic developed but only after a prolonged incubation amounting to 18 to 21 days.

The obvious criticism is that in using leaf tissue it is possible that the plants were inoculated with the "virus" as well as with the streptococcus-like organism.

Similarly cultures were made using 1/10 cc. of plant juice diluted 100 times and passed through a Chamberland F filter. Slight turbidity was apparent in four days at room temperature and infection occurred in plants inoculated.

All attempts to isolate and culture on gelatin or plant juice agar have so far failed although Ivanowski states that he was successful in obtaining some colonies.

EFFECT OF COLOURED LIGHT ON MOSAIC OF TOBACCO

In 1910 Lodewijks (83) attempted to ascertain the effects of colored light on mosaic of tobacco. He used plants which apparently had healthy lower leaves and which showed definitely diseased upper leaves. The upper and diseased leaves of the plants were enclosed in a hood of red cloth, orange cloth and blue cloth respectively, the basal leaves being exposed to daylight. The textile used was a coarse cotton bunting, and it was held away from the plant by wire hoops. The hoods remained over the diseased leaves for a period of from twenty to thirty days when they were removed and the leaves examined. Lodewijks as a result of his observations decided that red light diminished the disease, orange light checked it also slightly, but the blue light cured it. He postulated the development of an anti-virus in the apparently healthy leaves. The anti-virus was able to render the virus more or less impotent and especially so as the virus formation was diminished in the reduced light intensity to which the diseased leaves were subjected.

Chapman (48) repeated Lodewijks' experiments and in addition did what the latter omitted to do, viz. tested the effect on the disease by inoculation with juice from treated leaves into healthy plants. He found that diseased leaves kept under the red hood for twenty days still showed mottling as did also newly-grown leaves. The juice from these leaves was highly infectious. A week after removing the hoods the leaves showed the disease in "undiminished severity". A longer retention under the hoods did not modify the result and with the orange cloth the disease seemed if anything to be intensified.

In the case of the blue light the diseased parts of three plants were covered for twenty-five days, when a careful examination of the leaves was made. On two plants Chapman noticed only a slight curling on a few of the leaves, while on the third there was a slight mottling of two leaves. Two weeks after the removal of the hoods the two plants which showed only a slight tendency towards curling now showed a faint mottling of the leaves but the third plant developed distinct symptoms of mosaic again but not as seriously as before the treatment. The juice from all three, however, was infectious. He concludes that with blue light there is a suppression of leaf color variation which is more or less permanent in character but that the virus is still highly infectious in the "apparently normal fully recovered leaves". In neither case did these investigators consider the actual wave-lengths of light involved nor, what is perhaps a more important factor, the temperature inside the hoods.

The writer has carried out an extensive series of tests with a view to determining whether light of different group wave-lengths, with temperature maintained constant within ordinary limitations, may not affect plants suffering from mosaic. For this purpose tobacco was chosen because of the ease with which large numbers of plants can be grown and successfully infected. The procedure differed from that of Lodewijks and Chapman in several respects. Seedling plants were inoculated and at once placed under the various group wave-lengths of light. The plants chosen were of the same age, size, and as far as could be judged of the same vitality. Instead of enclosing only leaves showing mottling the whole plant was enclosed at once after inoculation and cloth hoods were replaced by bell-jars. Of these numbers 1, 2, 3, 4 and 5 were placed at the writer's disposal by Prof. F. E. Lloyd of McGill University, for which my thanks are here expressed.

The table below gives the wave-length transmitted.

No. of bell-jar	Color	Wave-length of light transmitted in millimicrons.
1.	Red	680 to 650
2.	Orange	650 to 480
3.	Blue	525 to 400
4.	Uranium	675 to 475
5.	Uviol	675 to 320
6.	Methyl green	560 to 520
7.	Methyl blue	575 to 425
8.	Erythrosin	675 to 575
9.	Gold orange	650 to 550

The experiments were carried on in the greenhouse where conditions are more under control than in the field, and in checking color differentiation in the affected leaves Ridgway's "Color Standards" were used.

The following tables give the observations with dates, etc., in the first series of tests in which the temperature was maintained at from 75° to 80° F. inside the bell-jars.

TABLE 1. Experiment set up June 8th, 1920.

Bell jar.	Date of 1st observation.	Results of 1st observation.	Date of 2nd observation.	Results of 2nd observation.
6	July 8	Weak growth Distinct mottling	Aug. 8	Mottling most pronounced. Excessive deformity.
7	"	Weakest growth Slight mottling but still noticeable.	"	Still weakest growth. Old leaves no change. New leaves mottled.
8	"	Strongest growth. Most mottling.	"	Mottling more pronounced in old leaves. New leaves very mottled but not so much as in No. 6.
9	"	Tall growth Mottling pronounced but not as in No. 8	"	Old leaves more differentiation. New leaves very mottled as in No. 8.

NOTE.—The plants were removed from the bell-jars on July 8th and from then till Aug. 8th they remained in the ordinary light of the greenhouse.

TABLE 2. Experiment set up Aug. 11, 1920.

Bell jar.	First observation.	Results of first observation.	Second observation.	Results of second observation.	Third observation.	Results of third observation.
6	Sept. 18	Weak growth Noticeable mottling.	Oct. 12	Still weak plant. Mottling more pronounced in three young leaves.	Oct. 20.	Most pronounced mottling deformity in new leaves.
7	"	Weakest growth. Mottling present but not marked.	"	Mottling still same. Slight deformity in two young leaves.	"	Increase in depth of color of dark green very slight.
8	"	Strongest growth and marked mottling. Deformity in 2 leaves.	"	No change.	"	Mottling more pronounced.
9	"	Tallest growth. Mottling as in No. 8.	"	More deformity and mottling than No. 8.	"	Mottling as in No. 8.

NOTE.—Plants removed from under bell-jars on Oct. 12.

TABLE 3. Experiment set up Nov. 2, 1920.

Bell-jar.	Date	Results	Date	Results.	Date	Results
6	Nov. 12	Very marked mottling in new leaves. Deformity also.	Nov. 25	Excessive deformity and mottling.	Dec. 5.	String leaf in two new leaves.
7	"	Mottling not marked but two new leaves deformed.	"	Mottling still not pronounced.	"	Mottling same but new leaves misshapen.
9	"	Whole plant pale green. Mottling and deformity in two leaves.	"	No change.	"	All new leaves mottled and deformed.

NOTE.—Plants were removed from under the bell-jars on Dec. 12 and examined again on Dec. 27. New leaves in No. 7 were badly mottled but old leaves were unchanged. In the case of No. 9 the whole plants had become distinctly darker and especially the darker areas of the affected leaves

TABLE 4. Experiment set up Aug. 11, 1920.

No.	Date.	Results.	Date.	Results.	Date.	Results.
5	Sept. 18	Mottling very marked.	Oct. 12	Mottling still marked. Plant flowering. Mottling in the corolla.	Oct. 20	Not considered.
4	“	Very stunted growth. Plant dark green. Mottling pronounced.	‘	Still stunted and darker than normal. Mottling very marked. Deformity in two leaves.	“	No change.
2	“	Mottling but not very marked. Three leaves deformed at tip.	‘	Plants weak. Two new leaves very deformed. Mottling more pronounced.	“	Differentiation more pronounced by the increased intensity in dark green areas.
1	‘	Weak growth. Slight deformity in two leaves. Mottling not marked.		Plants spindling. Color differentiation more pronounced.	‘	Increased intensity in dark green areas. Mottling and deformity in new leaves.
3	“	Weakest growth. Mottling only slight.	‘	Very weak growth. Leaves narrow and small. Mottling of speckled type.	‘	Mottling pronounced in two new leaves.

NOTE.—Plants removed from under bell-jars on Oct. 12 except No. 5.
Three plants under each of 1, 2, 3, and under each of 4 and 5.

TABLE 5. Experiment set up Oct. 20, 1920.

No.	Date	Results.	Date	Results	Date.	Results.	Date	Results.
5	Oct. 31	Mottling conspicuous. Deformity in three new leaves.	Nov. 12	New leaves mottled and corrugated.	Nov. 26	Mottling very marked.	Dec. 12	Plant flowering. Corolla mottled.
4		Dwarfed plant. Very dark green. Mottling marked. Deformity present.	"	Still very dwarfed and mottled.	"	Smallest plant. Mottling same.	'	Deformity more pronounced.
2	"	Pale green plants. Mottling present.	'	Tallest. Mottling more marked.	"	Deformity in one leaf.		Mottling more marked than No. 1.
1	"	As for No. 2.	'	Stouter than No. 2. Mottling same.	"	More deformity than No. 2.	'	Plants very pale green. Mottling less than No. 2.
3	"	Mottling showing but very slight. Youngest leaves misshapen.	'	Mottling slight.	'	New leaves deformed. Mottling still not marked.		As on Nov. 26.

NOTE.—Plants were removed from under the bell-jars on Dec. 12. By Dec. 27, Nos. 1 and 2 were more marked in their mottling by the intensification of the dark green areas. All new leaves were badly mottled and deformed. In No. 2 no change occurred in the old leaves but new leaves were distinctly more mottled.

In the second series tested from April 30, 1921 the results were in sufficient conformity with those of the first series for them to be tabulated en bloc.

TABLE 6.

Bell-jar.	Total Number of Plants	Results of observations 2 weeks after inoculation.	Effect when removed from bell-jars 10 days.
1	6	Plants pale, rather spindling. Mottling observable but not pronounced.	Mottling more pronounced. New leaves savoyed slightly.
2	4	Plants pale and weak. Mottling not pronounced.	Mottling more pronounced and mottled. New leaves deformed and mottled.
3	6	Poor growth. Mottling slight.	Mottling more pronounced in old leaves. New leaves deformed and mottled.
4	2	Dwarfed growth. Extreme mottling and deformity in 2 leaves.	Still dwarfed. No change in mottling.
5	4	Stunted growth. Mottling marked. Deformity in younger leaves.	Little growth. No change in mottling-intensity.
7	4	Slight mottling.	New leaves very mottled. Increase in intensity of mottling in older leaves slight.
8	4	Good growth. Mottling pronounced.	Mottling intensified in old leaves. New leaves deformed somewhat.
9	4	Tall growth. Mottling not very pronounced.	As above.

DISCUSSION OF THE RESULTS

In each case the plants under blue light exhibited less differentiation than any others, the mottling in some instances being discernible with difficulty. This was due, however, to a reduction in the intensity of green in the chloroplasts of non-hypoplastic areas and to the presence of more chlorophyll in the chloroplasts of the hypoplastic tissue. Freehand sections through both areas proved that there was tissue differentiation but not to such a marked extent as in bright sunlight.

After exposure to sunlight for a few days there was very little intensification of the mottling in the old leaves but all new leaves developed after removal from the bell-jars exhibited distinct differentiation, corrugation and frequently deformity. This was very pronounced in the case of plants in Table 5.

The expressed sap of the older, less differentiated leaves was highly infectious this result agreeing with that reached by Chapman (48). The fact that pronounced mosaic symptoms appeared in younger leaves which grew in the ordinary light of the greenhouse shows that the "virus" is still present in the tissues and able to become effective under suitable conditions. It is impossible therefore to believe that blue light is remedial. That no leaves showing ordinary mottling developed while the plants were under blue light does, however, tend to show that the effect of the "virus" is partially inhibited by blue light and to account for this at least three factors must be taken into consideration. Elongation of plants is at a maximum about the D line and it decreases towards the ultraviolet end of the spectrum and therefore blue light alone tends to reduce growth in the plant. Photosynthesis goes on most between B and C and the relative efficiency of the red and blue portions of the spectrum is as 100 to 14 according to Palladin. This ratio may be extreme but pyrheliometer values for orange yellow and blue bell-jars gave a ratio of 5 to 1 (MacDougal and Spoehr 153). Under blue light there is consequently a reduction in the photosynthetic activity of the plants which may affect the "causal principle" adversely. Finally there is a shading effect since the wave lengths transmitted are only those up to 525 Angstroem units in the case of blue light, so that all heat waves are cut off. These three factors influence the host directly and with our present ignorance of the nature of the "virus" it can only be conceded that the host, owing to its modified growth under blue light, is less affected by the virus than under favourable growth conditions.

Reference has already been made to the fact that unfavourable growth conditions tend to obscure the symptoms (pp.21-23). With the plants kept under red, erythrosin, gold orange, and yellow orange jars mottling was noticeable and freehand sections of leaves showed that there was tissue differentiation similar to that in diseased leaves grown in ordinary light but usually not quite so marked. It did not appear that these plants were more severely affected than plants grown in ordinary light although the juice from any part of them was highly infectious.

Excessive color differentiation combined with deformity occurred in those plants kept under green light (560-520 Angstroem units). This is in conformity with the generally accepted results of bactericidal action as far as the spectral colours are concerned, but with regard to the marked colour differentiation in the diseased leaves there is some difficulty. It is generally conceded that there are two maxima in the spectrum for synthetic activity, one in the orange-red and one in the blue (Kniep and Minder 152, Lubimenko 151, et al). Green is therefore a minimum synthesizing colour, and increase in dry matter has been found to be least in the green (Lubimenko). On the other hand Masulli (E. S. R. 23: 326, 1910) decided that green was favourable to the growth of some plants, and Schanz (Bot. Gaz. 1920) specifically states that potatoes grow better in green light than in yellowish. It is possible that tobacco, like potato, may be more suited, among coloured lights, by green than red or yellow.

With uviol glass blossoming time was advanced by some 7 to 10 days but vegetative growth was not favoured which is in accord with our knowledge of the effects of the ultra violet end of the spectrum, and mottling, with deformity, was marked.

Pronounced and permanent nanism with strongly marked mosaic mottling in the leaves which developed was typical of the plants under uranium glass.

As a definite check against the experiments performed by Lodewijks and repeated by Chapman, I arranged cotton hoods over the upper leaves of plants in two series. In the first the upper leaves already showed symptoms of the disease but five large lower leaves of each plant were not mottled. In the second red, orange and blue hoods were placed over the upper leaves of the plants immediately after one of the larger lower leaves of each plant had been inoculated. The hoods remained on from March 18th until April 10th, 1921. In the first series symptoms were reduced in that there was less clearly marked differentiation between darker and lighter green areas but they were still visible even in new leaves. In the second series symptoms were considerably reduced but after removal of the hoods newly developed leaves came on with definite symptoms.

The fact of major importance in view of what has previously been said is that during the day there was a generally higher temperature inside the hoods than outside. At 8.00 a. m. temperatures inside and outside were 70° F., at 10.30 a. m. that outside was on an average 73° F. but inside 80° F.; at noon outside 76°F. and inside 87° F.; at 1.30 p. m. outside 80° F. and inside 95° F. From 2 p. m. there was a gradual decline until by 5.30 p.m. both temperatures were equal at 70° F. Every day therefore during the experiments there was a period from about 10.30 a. m. until about 3.00 p.m. when the inside temperature ran on an average from 7 to 15 degrees Fahrenheit higher than outside and it is such an increased temperature during the day which brings about masking of symptoms.

EFFECT OF COLOURED LIGHT ON THE VIRUS OF MOSAIC OF TOBACCO

In the previous series of experiments complicated conditions obtain but on the whole the effect of the colored light was on the plants. It was impossible to decide whether the "virus" was directly affected or not. It is therefore worth while to determine whether colored light has any effect on the virus itself.

Three plants were chosen, plant A — suffering from typical mosaic but grown in the ordinary greenhouse light, plant B — one of those in Table 5 grown under blue light, and plant R — one of those grown under red light.

The sap was extracted from each plant, filtered and divided into three equal portions. Each portion was placed in a sterile 30 cc. shell vial, a layer of toluene added, and the vial tightly corked. One shell vial each of sap from plants A, B, and R was placed under bell-jar No. 1 so that they were entirely in red light, under bell-jar No. 3 in blue light, and under bell-jar No. 6 in green light. They were kept under the jars from Dec. 12, 1920 until Jan. 16, 1921.

On Jan. 16 forty-two plants of the same age, size, and apparent vigor were inoculated, each in two leaves with equal amounts of the "virus", as follows:—

Four plants with the virus of **A** kept under red light
“ “ “ “ “ “ **B** “ “ “ “
“ “ “ “ “ “ **R** “ “ “ “
“ “ “ “ “ “ **A** “ “ blue “
“ “ “ “ “ “ **B** “ “ “ “
“ “ “ “ “ “ **R** “ “ “ “
“ “ “ “ “ “ **A** “ “ green “
“ “ “ “ “ “ **B** “ “ “ “
“ “ “ “ “ “ **R** “ “ “ “
Three “ “ “ “ “ a typically diseased plant
“ “ “ tap water.

TABLE 7

Date	No. of plant.	RED LIGHT			BLUE LIGHT			GREEN LIGHT				
		Virus of Plant A.	Virus of Plant B.	Virus of Plant R.	Virus of Plant A.	Virus of Plant B.	Virus of Plant R.	Virus of Plant A.	Virus of Plant B.	Virus of Plant R.	Ordinary Virus.	Tap Water.
Feb. 16.	1	4 S(2 D)	1 M 2 S(2 D) 2 M	1 Sl 3 S(2 D)	2 S(2 D) 1 M	1 Sl 2 S(2 D) 2 Sl	1 Sl 2 S(2 D) 2 Sl	1 M 2 S(2 D) 2 M	1 Sl 3 S(3 D	1 Sl 3 S(3 D)	1 M 3 S(2 D)	O. K.
	2	3 S(2 D) 2 M	1 M 2 S(2 D) 2 M	2 S(2 D)	2 S 1 M	1 Sl 3 S(2 D)	3 S(2 D) 2 M	1 Sl 4 S(2 D)	1 M 3 S(3 D) 1 M	3 S(3 D)	1 M 3 S(2 D) 1 M	O. K.
	3	1 Sl 2 S(2 D) 1 M	1 M 4 S(2 D)	3 S(2 D) 1 M	3 S(3 D) 2 M	1 M 3 S(3 D) 1 Sl	1 M 3 S(3 D)	2 M 2 S(2 D)	1 Sl 4 S(3 D)	1 Sl 2 S(1 D) 1 M	1 Sl 4 S(3 D) 1 M	O. K.
	4	1 M 2 S(2 D)	1 M 2 S(2 D) 1 M	1 Sl 3 S(2 D)	1 Sl 2 S(1 D) 3 M	1 Sl 3 S(3 D)	1 Sl 3 S(2 D)	1 M 2 S(2 D) 2 M	1 Sl 4 S(4 D)	2 MS 2 S(2 D)		
Feb. 25.	1	+2 M	+2 M	+2 Sl	+1 Sl +2 M	+2 Sl	+2 M	+2 S	+2 M	+2 M	+2 S	O. K.
	2	+2 M	+2 M	+3 Sl	+2 MS	+3 Sl	+1 M	+2 M	+3 MS	+2 M	+2 MS	O. K.
	3	+3 M	+2 M	+2 Sl	+2 MS	+3 Sl	+2 Sl	+2 M +1 S	+3 MS	+2 MS	1 M	O. K.
	4	+3 M	+3 M	+2 M	+2 MS	+2 M	+2 Sl	+2 M	+2 M	+2 MS		O. K.

NOTE.—Sl = slight mottling, M = moderately mottled, MS = moderately severe, S = severe, D = deformity of leaves.

Thus 1 Sl
2 S(2 D)
2 M Signifies that the oldest affected leaf shows the mottling slightly, the next two are severely mottled and also deformed, and the two youngest leaves are moderately mottled.

TABLE 8

UNDER RED LIGHT						UNDER BLUE LIGHT						UNDER GREEN LIGHT						Ordinary Virus	
A.		B.		R.		A.		B.		R.		A.		B.		R.			
Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves	Value	No. of Leaves
20	6	18	7	15	6	15	6	13	7	15	7	22	7	17	6	17	6	22	6
22	7	18	7	16	5	16	5	16	7	18	6	21	7	25	8	16	5	22	7
17	7	22	7	18	6	22	7	17	6	16	6	20	7	26	8	17	6	21	7
16	6	14	7	18	6	21	8	18	8	15	6	18	7	21	7	20	6	—	—
75	27	72	28	67	25	74	26	64	28	64	25	81	28	89	29	70	23	65	20
Aver. 2.8		2.6		2.7		2.85		2.3		2.56		3.0		3.0		3.0		2.25	

The growing season in the greenhouse was just opening and the inoculated plants developed rapidly. They were checked regularly but in order to save space two dates only are given in Table 7.

It is not easy to give mathematical values to symptoms of disease in plants but in order to determine whether there was any effect on the "virus" it was decided to evaluate the symptoms as follows: Slight (SI) I, Moderate (M) 2, Moderately severe (MS) 3, and Severe (S) 4.

The reesults of applying these values to the plantsin Table 7 are given in Tables 8 and 9.

TABLE 9.

Light	Virus A.	Virus B.	Virus R.	Total	Average
Red	2.8	2.6	2.7	8.1	2.7
Blue	2.85	2.3	2.56	7.71	2.57
Green	3.0	3.0	3.0	9.0	3.0
Average	2.88	2.63	2.75		

These data permit the inference that there was attenuation of the "virus" in the vials kept under blue, red and green light in descending order as given. The average of infections thus obtained are:—for "virus" under blue 2.57, under red 2.7, under green 3.0, and with the check virus 3.25.

All three are below the check "virus", thus indicating an adverse effect on the "virus." This may be due to the effect of the light used or to the fact that the plants from which the "virus" was obtained were not so vigorous as those grown under ordinary light. The average of infection values for the "virus" from plant A, however, is higher than for that for B or R, the ratios being 2.88, 2.63 and 2.75 respectively. This is to be expected since there is a less active development of plants B and R under bell-jars than with plant A in the greenhouse. In the same way the “virus” of plant R is more effective on the average than that of plant B.

A second series was arranged on October 27, 1921, in order further to test the effect of light on virus from plants grown under ordinary conditions. Incidentally it was thought advisable at the same time to test the effect of freezing for a prolonged period, and also of filtration through a Chamberland F. Five medium-sized diseased tobacco leaves were cut up into shreds and ground thoroughly in ignited sand until a consistent paste was obtained. This was washed with 150 cc. of sterile water and immediately filtered through filter paper. Half the filtrate was then filtered by the suction of a tapwater pump through a Chamberland F. filter. The two filtrates, that through the Chamberland and that through filter paper, were then pipetted under sterile conditions into sterile test tubes so that approximately there were 15 cc. of diluted virus in each tube. A layer of toluene one-eigth of an inch deep was pipetted over each sample and they were then plugged with sterile cotton and sealed with paraffin wax. They were then arranged as follows:—

2 tubes (1 of each) in the dark in a Hearson Incubator at constant temperature of 21° C.
2 “ (1 “ “) in room at laboratory temperature.
2 “ (1 “ “) out of doors, temperature from 0°C. to — 8°C.
2 “ (1 “ “) green light (550-525μμ) at laboratory temperature.
2 “ (1 “ “) red light (700-610μμ) at laboratory temperature.
2 “ (1 “ “) blue light (485-425μμ) at laboratory temperature.

They remained under these conditions until January 12, 1922, when they were used as inoculum for 4 seedling plants in each case. The plants were growing well and each had three well-developed leaves at the time of inoculation. Each of the three leaves of each plant was inoculated by rubbing gently. The results and dates of observation are given in the following table:—

TABLE 10.

	January 31st				March 1st			
Plants.	1	2	3	4	1		3	4
Bf	Sl	Sl	M	?	1 SD 2 Sl	1 SD 1 M 2 Sl	1 D 2 M 1 Sl	1 D 3 M
Rf	?	M	M	Sl	1 SD 2 M 1 Sl	1 SD 3 M	1 SD 3 M	1 D 2 M 1 Sl
Gf	?	M	Sl	M	1 SD 2 M 1 Sl	2 SD 2 M	1 SD 2 M 1 Sl	1 D 3 M
Ff	?	?	?	?	?	2 M 1 Sl	1 M 1 Sl	1 M 2 Sl
Df	?	Sl	M	M	1 MS 1 D 2 Sl	1 SD 2 M 1 Sl	1 SD 2 MS 1 Sl	3 M 1 MS 1 Sl
Sf	?	Sl	M	Sl	2 Sl	2 Sl 1 SD	1 SD 2 M 1 Sl	2 M 2 Sl
Buf	M	M	M	Sl	1 M 2 SD	2 SD 1 Sl	2 SD 2 M	1 SD 2 M 1 Sl
Ruf	M	M	M	M	1 M 2SD 2 Sl	1 D 2 M 1 Sl	1 SD 2 SM 1 Sl	1 SD 2 M 1 Sl
Guf	M	M	M	M	1 M 3 SD	1 M 2 SD 2 Sl	1 M 2 SD 1 Sl	2 M 1 SD 2 Sl
Fuf	M	Sl	M	M	1 M 1 MS 1 Sl	1 M 2 SD	1 M 2 D	1 M 1 D 2 Sl
Duf	M	M	Sl	M	1 M 2 MS 1 SD	1 M 3 SD	1 Sl 2 M 1 SD	3 M 1 SD
Suf	M	M	M	M	3 SD	2 SD 2 M	2 SD 2 M	1 SD 2 M 1 Sl

NOTE. f = filtered through Chamberland F.
uf = filtered through filter paper only.
F = frozen, S = ordinary light, D = dark, B. R. G. colored light.

Evaluating the above results as was done with the previous table we have the following:—

TABLE 11. Value per plant.

Bf	Buf	Rf	Ruf	Gf	Guf	Df	Duf	Sf	Suf	Fl	Fuf
9.25	10.25	9.5	10.25	10	11.75	9.5	11.25	6	11.25	3	7.5

In each case it is seen that the "virus" filtered through a Chamberland F is less effective than that filtered through paper only. This is especially noticeable in the instance where the "virus" was kept in ordinary day light. It was anticipated that there would be an advantage in favour of that kept in the dark and this is so with the Chamberland filtrate but not with the paper filtrate. I can only account for the very low value of Sf by the possibility of a little toluene having been mixed with the inoculum, or by the fact that inoculation was not entirely successful in the ordinary sense as is frequently the case in mosaic. Freezing affected the "virus" materially, and markedly so where it was filtered through the Chamberland F. It is probable that the additional material present in Fuf protected the "virus." Regarding the effects of light of the red, blue and green groups the virus kept under the green again gave higher infection results but there is no difference of moment between the red and blue.

It is interesting to parallel these results with those arrived at by Laroquette (150) when studying the bactericidal effect of lights. Briefly stated, his findings were that in liquid media bactericidal action even of bright, protracted sunlight such as occurs in Algiers was slow, with diffused light negligible, and with coloured lights very slow action in descending order blue, yellow, red and green. Laroquette considered that green was next to darkness in its minimal adverse effect on bacteria.

In my results, allowing for the extra difficulties involved in infection of living plants, etc., it is remarkable that the results under green light are close in numerical value to those in the dark, and between blue and red there is but slight difference.

Taking into consideration therefore the effect of red and blue parts of the spectrum on the plant itself, and on the virus alone, it is clear that the contention of Lodewijks regarding the curative effect of blue light cannot be maintained, but rather it is the poorer growth under such illumination which masks symptoms inasmuch as the virus has not the opportunity of affecting rapidly-growing meristematic tissues. If temperature is also taken into consideration, the high temperature inside the hoods tends to mask symptoms even if the plants grow well.

INSECT TRANSMISSION, SEED INHERITANCE AND CROSS-INOCULATIONS

INSECT TRANSMISSION

In connection with insect transmission of plant diseases a valuable review of our knowledge is given by Rand and Pierce (107) but it is advisable here to summarize information pertaining directly or closely to mosaic transmission.

Allard (1) experimentally demonstrated that *Myzus persicae* may become an active carrier of tobacco mosaic in the greenhouse and *Macrosiphum tabaci* in the field. The writer can corroborate the former statement inasmuch as *Myzus persicae* transferred from capsules of diseased plants to healthy seedlings successfully transmitted mosaic in three cases out of four, the disease becoming evident eighteen days after the aphids had been placed on the plants. It has been impossible to do this with older plants because of the abundance of glandular hairs on tobacco leaves.

Schultz and Folsom (118) found that mosaic of potato is transmitted by *Macrosiphum solanifolii* and *Myzus persicae* from potato to potato. In the experiments large numbers of aphids were used (130—170 per plant) and it would be of interest to know what is the minimum number required for transmission. It is possible that a proportion of the aphids feeding on diseased plants may not imbibe the disease-causing principle or not sufficiently to transmit it. A statement of the authors is noteworthy:

> "The greenhouse experiments that have been described showed that both spinach aphids and potato aphids . . . can transmit potato mosaic from diseased to healthy plants and that this transmission sometimes may not be followed by the development of mosaic symptoms until the tubers produce the second generation of plants."

Brandes (28) finds that sugar cane mosaic is transmitted by *Aphis maidis* and that this aphid also transmits mosaic of sorghum and corn. In fact

"It has been proved that the virus of corn mosaic is identical with that of sugar cane and sorghum mosaic, so that even if it is found not to be seed borne, perpetuation of the disease in the perennial grasses would explain its appearance on corn in the spring.

Doolittle (48) and Jagger have demonstrated that *Aphis gossypii* is instrumental in the production of epiphytotics of cucumber mosaic and Doolittle states that infection occurred when only 3 aphids were placed on each plant.

Ordinarily, however, the aphids are not present in cucumber fields to such an extent as to cause frequent epiphytotics and under such conditions *Diabrotica vittata* and *Diabrotica duodecimpunctata* transmit the causal principle readily.

Similarly spinach blight, *Microsiphum solanifolii* (Ashmead) was the chief carrier of the infective virus (McClintock and Smith, 91). These authors state that

"The infectious entity of spinach-blight was found to be transmitted by virus-bearing adult aphids to their off spring. It was also found that although aphids were reared on lettuce for four generations consecutively, yet a few of the fourth generation were virus bearers and produced infections when they were transferred to healthy spinach."

Myzus persicae transmit mosaic of lettuce (Jagger, 71) and mosaic of turnip to turnip and mustard, of mustard to turnip and mustard and of Chinese cabbage to mustard (Schultz, 117).

CLOVER MOSAICS

The present writer (43) has shown that *Macrosiphum pisi* (determined by Dr. E. M. Du Porte) can transmit mosaic from red clover to red clover, alsike clover, white clover, crimson clover and trefoil clover. In carrying out the tests from February to September, 1921, in the greenhouses at Macdonald College plants were segregated in large insect cages in order that there could be no possibility of accidental infection. Regular fumigation was carried out so that white fly and other insects could not become a factor in the experiments. All the plants used were grown from healthy seed and they were sufficiently well-grown at the time of each experiment to guarantee their being healthy. The following table indicates the results.

TABLE 1. Infection by Macrosiphum pisi Kalt.

Date of Inoc.	Source of Aphids.	Plant Inoc.	No. of Plants	Date of first Symptoms.	No. of Plants Infected.
February 25	T. prat.	T. prat.	8	March 15	5
March 9	"	"	7	March 30	6
March 12	"		5	April 2	3
April 1		"	7	April 17	4
March 1	"	T. Hybridum	9	March 23	5
April 1	"	"	3	April 18	2
April 3			3	April 18	2
April 12	"	"	5	May 2	2
May 20	"		6	June 7	4
June 1		M. lupulina	8	June 20	5
June 1		T. repens	7	June 23	4
August 3		T. incarnatum	5	Aug. 27	3
August 3		M. alba	8	Sept. 30	0
March 20		M. sativa	1	April 17	1

NOTE. 10 aphids were used on each plant.

Checks were run with the experiments using aphids from healthy red clover plants in each case on two plants of the varieties tested. In no instance did the aphids from healthy red clover cause any symptoms of mosaic in the check plants to appear.

No attempt was made to determine the minimum time required for feeding on diseased plants in order that aphids might become carriers, nor the minimum time required for infection of healthy plants. Work is in progress on that phase and also as to progeny infection among aphids.

It will be noticed that in one case mosaic symptoms developed on alfalfa. The appearance was typical and all young foliage became streaked and somewhat malformed. Unfortunately the summer was so hot that it was impossible to replicate this experiment satisfactorily and further work will be done on it.

RASPBERRY MOSAIC

During the summer of 1920 mosaic of raspberry was transmitted by aphids. On June 12th seven healthy plants were inoculated by placing on well-grown leaves aphids from diseased plants. By July 23rd new growth was obviously mosaic-diseased in three plants. The plants were dug in late autumn, left in the cold until January 12th and then planted in the greenhouse. By March 10th they were in blossom and every leaf exhibited typical mosaic symptoms. The aphids were not determined owing to unforeseen circumstances and the writer was not able to collect any during the summer of 1921 but it is highly probable that they were *Aphis rubiphila.*

The relation of aphids to mosaic of potato, raspberry, clovers, lettuce, etc., becomes of prime importance when one considers diseases of a somewhat similar type. Thus aphids have been found to transmit potato Leafroll and *Eutettix tenella* carry the causal principle of curly top of sugar beet.

It is possible that plants may suffer from protozoan diseases as is the case with man and lower animals. In connection with this mention may be made of a recent article by Franca (57) in which he found a flagellate, *Leptomonas davidi,* occurring in the latex of

Euphorbia peplus and *E. segetalis* in Portugal and transmitted by the sucking Coreid *Stenocephalus agilis,* Scop. In the insect giant forms are found such as do not occur in the latex of *Euphorbia sp.* Later minute forms appear which invade the salivary glands and infect *Euphorbia sp.* on which the insect feeds.

Previously in 1910 Lafont demonstrated that *Leptomonas davidi,* Lafont caused "flagellosis" in the latex of *Euphorbia sp.* and that the alternate host was *Nysius euphorbiae* Horvath.

SEED INHERITANCE OF MOSAIC

The importance of inheritance of mosaic through the seed has naturally attracted considerable attention.

Westerdijk (141) in Holland believes that tomato mosaic is seed-transmitted but the work of Allard (5) and others on this continent discounts this view. He states that "although in the writer's experience no evidence of embryonic infection in the seed has even been obtained, it would not be safe to assert that this never occurs. However, all evidence at hand indicates that it must be of very rare occurrence."

In working with tomato mosaic the present writer was on the lookout for any instance of definite embryonic infection. During the course of the investigations approximately six thousand plants have been closely observed and not a single case of embryonic infection has been noticed.

The earliest sign of mosaic appeared in the third true leaf of each of four plants grown in the greenhouses during the winter of 1919. To account for this it is possible that the infecting principle is carried by pulp which necessarily adheres to the seed coat. The time elapsing between obtaining the seed from field grown tomatoes and planting in the greenhouse is short and the virus would doubtless still be effective. In most cases the cotyledons bring up the seed coat above ground attachel at the tips. In many instances the cotyledons are held at the tips by the seed coat for some days and the tension may injure the tissues of the cotyledons near the edges of the seed coat. The growing plants are watered regularly and if pulp from a diseased fruit happens to be adhering to the surface of the seed coat under these conditions infection is possible, so that cotyledon infection might account for symptoms showing definitely in the early leaves as above mentioned.

With tobacco, petunia, pepper, black henbane, ground cherry, and thorn apple no records of seed inheritance are known. Similarly there is no account of seed inheritance of mosaic of potato probably because this test has not been applied, to the writer's knowledge. The Solanaceous group of plants therefore comprising highly susceptible species is noteworthy for its lack of seed inheritance of mosaic.

The contrary is the case with the Leguminous group of plants susceptible to mosaic for in this group seed inheritance appears to be common. A study is being made to determine the reason for the contrast in these cases.

Stewart and Reddick (111) found that seed from diseased pea bean plants gave rise to diseased seedlings; McClintock (89) ascertained the same fact for Lima beans; and Gardner and Kendrick (59) proved that seed transmission occurs with Soy beans.

The writer, in conjunction with Dr. G. P. McRostie (44) has determined that seed transmission of mosaic is of frequent occurrence with clovers as the following experiments indicate.

In one of the clover plots at Macdonald College there are 1075 hills with one plant each of *Trifolium pratense* and in September, 1920, I found that 47 per cent were suffering from mosaic. *Macrosiphum pisi* Kalt were fairly plentiful during that summer and it was anticipated that there would be an increased percentage of diseased plants during the 1921 season. Counts made on June 30th, 1921, showed that 779 plants were definitely diseased, 73 were doubtful and 23 were healthy, thus giving 91 per cent mosaic, 6.8 per cent doubtful and 2.15 per cent healthy.

Twenty-two representative mosaic-diseased plants were selected and the seed obtained carefully counted. They yielded 1443 seeds or 65 seeds per plant. Twenty-two representative healthy plants yielded 10,566 seeds or 484.8 per plant.

Beds in the greenhouse were sterilized by the formaldehyde method; the house was fumigated thoroughly. On October 5th, 1921, the

TABLE II. **Seed Inheritance of Mosaic in T. pratense.**

Plant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Seeds	62	95	85	43	15	60	102	15	34	95	105	45	80	41	115	46	95	115	30	45	65	55	1443
Germ	3	5	18	5	3	6	12	0	4	4	7	4	7	4	40	5	26	11	4	5	10	3	186
Mosaic	1	—	3	3	—	1	2	—	—	—	1	2	1	1	8	1	2	1	1	—	—	1	24
Doubtful	—	3	1	—	3	—	8	—	4	4	2	—	2	—	5	—	4	2	—	2	2	—	87
Healthy	2	2	14	2	—	5	7	—	—	—	4	2	4	3	30	4	20	8	3	3	8	2	125

1443 seeds from diseased plants and 500 seeds from healthy plants were sown individually. In the case of the healthy seeds plants grew giving germination of 75 per cent and in no case was there any sign of mosaic in the seedling.

The results with the diseased seed are given in Table 11.

The decreased germinating power of seed from diseased plants is very marked and it is to be noted, as the counts were made 10 days after germination (November 1st, 1921), many plants in the doubtful column later showed mosaic. The counts were made at that interval, however, in order to obviate any possibility of accidental infection.

A further fact bearing on the results is that under greenhouse conditions of regulated moisture and temperature symptoms are likely to be masked to a considerable extent. It is, therefore, suggested that the results are on the conservative side.

An outside test was conducted simultaneously but owing to the sudden oncoming of snow this test will be repeated in the spring. Commercial seed from St. Rosalie, Que., was sown on October 5, after possibility of aphid infection was past. On November 10th approximately 10,000 seedlings were examined and 5 were found to show distinct symptoms of mosaic.

Of 210 seeds of commercial *T. hybridum* planted under the same conditions in the greenhouse on October 5th, 34 germinated of which 31 were healthy 2 doubtful and 1 diseased on November 1st. By January 1st, 10 more had germinated of which 2 were diseased.

On December 2nd commercial seed from St. Rosalie, Que., was planted in the greenhouse and out of 4,000 plants (95 per cent germination) 5 were doubtful and none diseased.

On the same date mixed seed, from the plots containing both healthy and diseased plants was planted. Out of 800 plants which grew 9 were distinctly diseased.

Seed from diseased sweet clover plants (*Melilotus alba)* were collected in the vicinity of the campus on September 20th, 1921. They were sown on December 1st without scarifying as it was not possible to be certain what effect scarifying might have. By January 5th, 1922. twenty-eight plants had grown of which 4 exhibited definite mosaic symptoms.

Mosaic of ***Pisum sativum*** is severe on Golden Vine and 70 seeds of this variety from mixed, healthy and diseased, plants were sown on December 3rd, 1921. By January 5th, 1922, 38 had germinated and 29 were markedly diseased, the remainder being healthy.

Other varieties tested afforded the following results: Arthur, 13 diseased out of 22 germinated; Canadian Beauty, 8 out of 24; White Marrowfat, 2 out of 17; Chancellor, 2 out of 19; and Grass Pea, 1 out of 20.

Efforts to germinate cultivated raspberry seeds have not been very successful but the evidence indicates that raspberry mosaic is not seed-inherited (p. 94).

On the other hand mosaic of ***Hippeastrum sp.*** would appear to be seed-inherited since in two specimens out of eight grown from seed in the experimental greenhouses at Macdonald College a pronounced mosaic condition appeared.

CROSS-INOCULATIONS

Whatever view may be held regarding the etiology of mosaic diseases it is necessary to know to what extent the causal principle of any given mosaic may bring about infection in healthy plants of other species, genera, etc.

It is now accepted, as a result of the work of Clinton (33, 34), Allard (5, etc.), and others that the mosaic of ***Nicotiana tabacum*** is transmissible to other varieties of ***Nicotiana*** (with exceptions noted later) to ***Lycopersicon lycopersicon, Petunia violacea, Physalis sp. Datura stramonium, Datura tatula, Solanum nigrum, S. Carolinense, Capsicum annum*** and ***Hyoscyamus niger.*** Westerdijk (141) did not find that mosaic was transmissible between tobacco and tomato and it is recorded that Ivanowski (69) and Koning (77) could not transmit tobacco mosaic to ***Hyoscyamus niger.***

In my work with solanaceous hosts I found it possible to begin with tomato mosaic, transmit it to tobacco. petunia, black henbane, and pepper and to cross-infect any of these from the other with the exception of pepper. In this case infection from pepper to tobacco or black henbane was usually not difficult but from pepper to tomato was never very satisfactory.

I attempted many times to infect *Solanum tuberosum, Solanum melongena* (Sec p. 8), *Atropa belladonna, Lycium vulgare, Salpiglossis sinuata* and *Schizanthus sp.* from tobacco but did not succeed.

Nishimura (100) in recent work has added *Solanum aculeatissimum* to the list of plants inoculable from tobacco and shows also that *Physalis alkekengi* can act as a carrier without showing symptoms. Similarly in this latter respect scions of *Nicotiana glauca* and *N. glutinosa* grafted on diseased stocks of *N. tabacum* can carry the virus without manifest symptoms in the case of *N. glutinosa* and with but slight symptoms in *N. glauca* (Allard, 8). Not all species of *Nicotiana* have the same mosaic since experimental evidence indicates that mosaic of *N. viscosum* is not communicable to *N. tabacum,* tomato, nor pepper, but was communicable to *Datura fastuosa* which cannot be infected from *N. tabacum* (Allard, 7).

It may therefore eventuate that two kinds of mosaic occur among Nicotianas, etc. and potato mosaic may be a third distinct type, making three for Solanaceae. That potato mosaic may be distinct is shown by the difficulty of infection of other plants from it or of it from others.

One case is reported where infection occurred in a seedling potato infected from tobacco (Clinton, 34) and by grafting a healthy scion of tomato on diseased potato the scion became diseased although not typically (Krantz and Bisby, 146).

Among the Cucurbitaceae, mosaic is transmissible between cucumbers, gherkins, muskmelons, gourds and wild cucumber. It is found, however, that watermelon, citron and other species of Citrullus (except *C. vulgaris*) are resistant (Doolittle, 48) although Jagger (48) obtained infection in one case on watermelon and Freiberg (58) illustrates the disease on citron.

Cucurbit mosaic has been transmitted to *Lobelia erinus gracilis* and to *Helianthus debilis* by Jagger (Doolittle, 48) indicating that in some cases the disease may pass to hosts outside the Cucurbitaceae although too much emphasis must not be laid on such exceptional cases unless they are very definitely possible of transmission by insect agency.

The Gramineae constitute another large group and Brandes (27) has demonstrated that in sugar cane, sorghum, crab-grass, foxtail, and Panicum the infectious principle is the same. Similar symptoms occur on millet, corn and rice but these plants are more difficult to infect. Golden Bantam sweet corn appears to be immune under ordinary conditions (Brandes, 29).

Three cases of mosaic are now known in the Cruciferae, viz. those of Chinese cabbage, mustard and turnip (Schultz 117) and these are inter-transmissible.

Among the Leguiminoseae there is a large number of susceptible plants. Reddick and Stewart (110) have successfully cross-infected mosaic from *Phaseolus vulgaris* to *P. lunatus macrocarpus, P. acutifolius latifolius, Vicia faba,* and they give an extensive list of varieties of *P. vulgaris* which are susceptible.

Recent work has been largely concerned with mosaic of the various clovers (Dickson, 41a; Elliott, 52; McLarty, 93) and the account of my work in cross-inoculating by aphids has already been given. Similar results were obtained by juice inoculations made by rubbing leaves of plants grown under protected conditions in the greenhouses at Macdonald College from February to September, 1921. The period of incubation varied from 10 to 15 days being longer at cooler temperatures.

Elliott (52) cross infected *Trifolium pratense, Melilotus alba, Medicago arabica,* and *Vicia faba,* but could not infect *Trifolium repens.* In my work I was able to infect *T. repens* (Pl. III, Fig. 14) and in addition *T. hybridum, T. incarnatum* and *Medicago lupulina.* In one instance there appeared to be successful inoculation of *Medicago sativa* by means of aphids. I was not able, however, to infect either of the sweet clovers from any of the other clovers.

The possibility of cross-infection between beans, field peas and the clovers is a problem of some economic importance and I have made numerous attempts to obtain positive evidence of such cross-infection. Elliott (52) was able to cross-infect certain clovers from *Vicia faba* but the occurrence of mosaic of Windsor bean in Quebec rendered it inopportune to perform any inoculations. Cross-inoculations have repeatedly been made between *Phaseolus vulgaris, Pisum sativum*, *Trifolium pratense, Trifolium hybridum* and *Melilotus alba.* Between *Phaseolus vulgaris* and the clovers results are so far negative and in only one case is there a positive result with infection from *Pisum sativum* in which a plant of *Trifolium hybridum* became typically diseased eleven days after inoculation. With sweet peas I was successful in infecting four plants out of twenty-three from *P. sativum* but no positive results were obtained by inoculating from beans.

While it would appear extremely probable that cross-infection among the Leguminoseae generally should occur the results so far can be considered definitely positive only among clovers.

MOSAIC OF RASPBERRY

HISTORICAL SUMMARY

Of all raspberry diseases that commonly known as "yellows" or "curl" is the most serious in Quebec. It attacks cultivated red raspberries of which Cuthbert is the most susceptible and the writer has found wild red raspberries suffering markedly from the disease from the Island of Montreal to the Gaspé Peninsula.

Green (61) in Minnesota was the first to report its occurrence under the name "Leaf Curl" in 1894 and again in 1895, although Detmers (39) in 1891 had described a similar disease but had ascribed its cause to bacteria. From the description given by Detmers there is no doubt that the disease in question was "yellows".

It was reported from New York as raspberry "yellows" by Stewart and Eustace (131) who differentiated it from raspberry cane blight known to be caused by *Leptosphaeria coniothyrium* (Fckl.) Sacc. These authors suggested that the disease is akin to peach yellows and give the following account of the symptoms.

> "On fruiting canes the fruit-bearing laterals are dwarfed, often to one-half their normal length. The leaves are small, curled slightly downward at the margins and faintly mottled with yellow. Some of the berries dry up without ripening and those that ripen are undersized and insipid. Much of the foliage withers at the same time. New canes, for the most part, are not seriously checked in growth although their foliage is usually more or less affected. The foliage on new canes does not wither and there are rarely to be found any dead spots or areas.

"Yellows" attacked especially the Marboro variety in Colorado (Paddock, 104) and it was more severe where the water table of the soil was higher. That poor drainage and poor soils are factors conducing to susceptibility is the view of Lawrence and Melchers (95).

The below-mentioned extracts give the symptoms of Curl or Yellows according to Melchers.

"One of the more striking symptoms of this disease is the stunted or dwarfed appearance of the plant. A withering or blighting of the canes or leaves never occurs in the case of this disease.

"The canes are short, and when the fruiting laterals are formed the plant has a compact, bushy appearance. The internodes both in the canes and in the laterals are very short. We may have apparently healthy and diseased canes arising from the same crown. Premature flowering of the current year's growth is not uncommon, terminal inflorescence being frequently preseent. Diseased sprouts emerge from the soil with a sickly, pale yellowish-green color, the leaflets being small and more or less curled. The writer has observed such sprouts arising at a distance of three feet from the parent crown. As these canes grow older, the leaflets become darker green and noticeably revolutely curled.

"The most striking characteristic of the diseased leaflet blade, is the arching upward of the intervenal tissues, which cause the vascular system to appear sunken. It is this uneven expansion of tissues which brings about the revolutely curled condition of the diseased foliage. During the summer months the foliage may acquire a mottled appearance, at first a light yellow, gradually changing to darker shades of green and yellow and eventually transforming into a reddish-bronze hue. The severity of curl and variations and intensities of color depend very greatly upon soil and climatic conditions. An abundance of rain is unfavorable to the development of the above symptoms, while hot and dry weather produce the more conspicuous cases. All diseased foliage, besides being curled and mottled, is considerably smaller and never attains its natural size. In September or October it is not uncommon to find considerable mottled foliage; the spots may vary from a yellowish tinge to a bronze, in many cases not unlike mosaic disease in their color, size, shape and location with reference to the vascular system.

"The berries mature from ten days to two weeks earlier than the normal crop. They are small, often deformed, lighter in color than the normal berry, and when apparently ripe are bitter, later becoming insipid. If allowed to remain on the cane until they become "dead ripe" they acquire a slight flavor, which is, however, far from pleasant."

It is clear that although this author mentioned mosaic-like symptoms he did not consider that there was a raspberry mosaic as distinct from "Curl". Nor is it clear in the work of any previous investigator whether "Yellows" is interchangeable with "Curl", whether it includes "Curl" or is a separate disease. Melchers certainly considers the first to be the case as is indicated by the title of his article (95).

The writer, in a paper (42) read before the Canadian Branch of the American Phytopathological Society at Guelph on December 10th, 1920 presented tabulated evidence to show that there is a distinct Mosaic of Raspberry as separate from "Curl". As to whether it has been in the past included in "Yellows" depends upon the interpretation generally applied to that term. It would seem preferable to use the name "Yellows" as a synonym for "Mosaic" in this case and thus have two diseases "Mosaic" and "Curl" of raspberry.

Mosaic of raspberry is very common in Quebec and reference has already been made to the part played by aphids in speading the disease (p. 81). The writer has not been successful to date in ascertaining whether aphids function in disseminating the disease among the wild varieties of raspberry.

MACROSCOPIC CHARACTERISTICS

Diseased plants exhibit characteristics remarkably similar to those of tobacco, pea bean, black henbane, tomato, etc., which are suffering from "mosaic". The leaves are mottled with dark and light green areas which vary in size and shape. In some cases the light areas are small and numerous; in others they are larger taking in considerable parts of the inter-venal tissues (Pl. V., Fig. 4). Again the whole leaf with the exception of a small part may be light green. But the leaves are never altogether yellow nor altogether dark green as in "Curl". The lighter areas are retarded in growth and obviously thinner while the darker areas grow so that excessive corrugation may occur. This is not the case where the light areas are small and numerous.

The differences in the thickness of the lamina are easily seen and if a leaf be held up to the light such differences in thickness and color are still more noticeable. The mottled appearance is evident early in the season and shows in the youngest stages of the leaves. From the time the leaf is fully grown, as far as the diseased leaf is able to become so, no further modifications occur except maturation changes.

The leaflets are as a rule smaller and frequently deformed. The tendency is for the leaflet to become narrower the more pronounced the mosaic condition becomes. In the deformed leaflets one-half may be normal in shape, although subnormal in size, whereas the other half is depauperate. The petiole is not generally reduced in length so that the leaves tend towards a spindly type. This again is remarkably similar to the leaf of a mosaic-diseased tomato plant. On the other hand with "yellows" or "curl" the chiief characteristic of the leaf aside from its coloration and shape is the shortness of the petiole giving the leaf a most compact and "unraspberry-like" appearance.

While the mosaic-diseased canes are somewhat dwarfed and weaker than normal canes they cannot be mistaken for those suffering from "yellows" or "curl". The amount of dwarfing varies from hardly any to approximately thirty per cent in ordinary cases of the disease and when more severely affected the dwarfing is of the spindly type. With "yellows" or "curl" the canes are stunted to a much greater extent and are not spindling but stout. The writer has observed many cases of mosaic occurring on canes six feet in height although as a rule they are from three to four feet. "Yellowed" canes rarely reach a height of more than two feet to two feet six inches when severely affected.

The plant as a whole is not bushy but normal in general shape although subnormal in size and it is lighter green than normal in general coloration. A bushy appearance is sometimes noticeable because of the dwarfed, rather spindling fruiting branches on two year old canes.

Affected plants do not bear the normal quantity of fruit but the fruit actually borne does not differ perceptibly from that of a healthy cane. The writer has not been able to accumulate sufficient data to warrant a conclusion as to the depreciation in crop by mosaic but preliminary tests tend to show that there is a definite and increasing loss in yield after two years.

The following tabulation of macroscopic characteristics will give a clear presentation of the features upon which the writer bases his differentiation between "mosaic" and "curl" of raspberry.

MOSAIC.	"YELLOWS" or "CURL"
1. Leaf mottled.	1. Leaf either altogether yellow or dark green only.
2. Mottling permanent.	2. No true mosaic mottling. Intervenal areas may degenerate.
3. Leaflets tend to be narrow in lamina and therefore are long in appearance.	3. Leaflets tend to be short and therefore broader in appearance.

4. Corrugations only in darker areas, the lighter areas being retarded in development. Corrugations quite irregular therefore.	4. Corrugations in both wholly yellow and wholly dark green leaves identical. The intervenal areas are curved upwards giving the veins a sunken position. The leaf looks as if it could be folded like a fan. Corrugations fairly regular.
5. Petiole not excessively reduced in length especially between leaflets.	5. Petiole excessively reduced in length especially between leafets.
6. General appearance of leaf spindly.	6. General appearance of leaf compact.
7. Canes not excessively dwarfed as a rule.	7. Canes excessively dwarfed.
8. General appearance of plant normal except in severe cases when all growth is subnormal.	8. General appearance of plant always very dwarfed and compact with stout canes.
9. Berries do not mature earlier.	9. Berries mature from a week to two weeks earlier than normal.
10. Berries approximately normal in size, shape and taste.	10. Beries small, often deformed and either insipid or bitter.

SEED INHERITANCE

It would appear that mosaic of raspberry is not inherited through the seed. Raspberry seeds are difficult to germinate and out of about one hundred seeds from a severely affected plant two seedlings which were perfectly healthy grew. One has since died but the other, now eight months old, is still without a sign of mosaic. Seeds from wild raspberries germinate more easily but out of many trials no case of a diseased seedling has yet been found. In conversation Dr. E. C. Stakman and Dr. M. J. Dorsey state that under Minnesota conditions mosaic of raspberry is not inherited in the seed thus confirming the writer's result in Quebec.

INOCULATION EXPERIMENTS

1. *By hypodermic needle.*

Ten healthy plants were inoculated on June 15, 1920, with juice from mosaic-diseased leaves and canes which had been filtered once through filter paper.

Ten other healthy plants were inoculated with juice which had been filtered through a Chamberland F filter.

In each case inoculation was made by means of a fine hypodermic needle into a vein in the hope of forcing the inoculum into the phloem tissues. In one plant of the first group slight mosaic symptoms appeared during the first week of August, 1920, and in June, 1921, this plant was distinctly diseased. Although these plants were distant from diseased plants they were not screened from aphids and the possibility of aphid inoculation must be borne in mind.

2. *By bruising leaves and applying juice.*

Many attempts were made under varying weather conditions during 1920 and 1921 to bring about infection by rubbing in juice from diseased leaves and canes and in three cases mosaic developed in inoculated plants.

3. *By root inoculation.*

During the summer of 1921 experiments were conducted to determine whether inoculation via roots was possible.

Twelve plants were used and in each case a main root was carefully exposed, washed and sterilized with 95 per cent alcohol where it was to be severed. It was cut through under sterile conditions and a thistle funnel with rubber connection attached and sealed on the severed end. The inoculum was poured into the funnel and a layer of toluene floated on top. Air was removed by the insertion of a sterile needle between the rubber tubing and the root when pouring began and the joint was then dried and sealed with hot wax.

Two plants in each case were thus inoculated with distilled water, juice from healthy plants, juice from mosaic-diseased plants, juice from plants with curl of the dark green type, and juice from plants with curl of the pale yellow type.

In no case were satisfactory results obtained.

4. *By pruning.*

Circumstantial evidence would seem to indicate that pruning may be a method of inoculation. It is a fact that the progress of the disease along rows of plants in large areas is more or less regular, the disease gradually extending along a row from a centre of inoculation. Experiments are being planned to determine whether infection may result from pruning under ordinary conditions.

SUMMARY

A list, complete to date as far as the literature available to the writer is concerned, is given of plants known to suffer from mosaic diseases. This includes several new mosaic diseases determined by the writer, viz:—Mosaic of ***Pisum sativum, Trifolium hybridum*** and ***Medicago lupulina.*** A single instance of what appears to be mosaic is mentioned as occurring in ***Medicago sativa.*** Raspberry mosaic is differentiated from a complex of diseases variously known as "Yellows" or "Curl", in a special part of the paper.

Details of the symptoms of the plants studied in the course of these investigations are given, including the new ones above-mentioned. The finding of striped stems in diseased tobacco, and of many cases of excessively mottled tomato fruit is noted, as is also the occasional occurrence of mottling on pepper fruits and bean pods.

Symptoms are modified by conditions affecting the growth rate of plants. Cramped root systems, too low temperature or poor soil or insufficient light check rapid growth in the plant and in such cases marked mottling, savoying, etc., are absent. A transfer to favourable growth conditions brings about the occurrence of obvious symptoms in new leaves. Temperature is an important factor in this respect and infected plants at low temperature showing no definite signs of disease removed to temperatures about 70° to 80° F. exhibit marked symptoms in new leaves, but if maintained at still higher temperatures, 90° F. and above, the symptoms are again masked in new leaves.

The amount of inoculum, and its virulence, affects the symptoms inasmuch as leaves developing immediately after heavy infection of seedling plants are deformed and savoyed, while frequently those growing later show ordinary, or perhaps only slight, infection. It would appear that, as above, growth is so adversely affected that there is a tendency toward masking the symptoms in the latest developed leaves.

In diseased plants there is a reduction in the set of seed and in the germinating power of that seed which does mature. This is shown in the case of *Vicia faba, Trifolium pratense* and *Pisum sativum.*

Detailed studies of the pathological histology are given with the following plants:—tobacco, tomato, potato, petunia, black henbane, pepper, raspberry, sweet pea, Canada field pea, Windsor or broad bean, pea or Kidney bean, red, white, Dutch, crimson and trefoil clovers. It is shown that in all cases the following leaf histological modifications occur:

1. Hypoplasia in lighter green areas manifest especially in the palisade tissue but present also in the spongy mesophyll cells.
2. Reduction in the intercellular space volume of these areas by reason of the more regular arrangement of the cells.
3. Reduction of the chlorophyll content of these cells because of fewer chloroplasts, less chlorophyll per plastid, breaking down of plastids or the general coalescence and degeneration of the plastids.
4. The presence in these cells of secondary contents derived from the degenerated plastids and possibly cytoplasm.
5. The small hyaline bodies found in cells of diseased chlorenchyma are apparently derived from fragmented plastids and when found they are in a state of rapid motion. The 106 reason for this has not been determined but it may be the result of the presence of invisible, rapidly moving bodies. In other cells agglomerated and degenerated plastids line the lower walls and such cells are moribund if not dead.
6. Reduction in the area covered by each epidermal cell because of hypoplasia below. Growth takes place, however, so that either the epidermal cells over hypoplastic chlorenchyma are deeper than normal or they develop trichomes to a greater extent numerically than normal.
7. The gradation from healthy through slightly diseased to severely affected is indicated by the extent of hypoplasia above-mentioned.

8. In leaves, other than those slightly affected, the transition from hypoplastic to non-hypoplastic tissue is sudden, occupying a linear space of three or four palisade cells.
9. When adverse conditions arise such tissues as are most hypoplastic may die and thus give rise to brown-flecked leaves.
10. Darker green areas are in ordinary cases slightly hypertrophied and markedly so where savoying occurs. The hypertrophy is pronounced in the palisade tissues.
11. Intercellular space volume is greater in such hypertrophied tissues than in healthy and considerably greater than in hypoplastic.
12. Chlorophyll content is greater than normal not only because of the increased number of cells but because of an increased amount of chlorophyll and number of chloroplasts per cell.
13. Epidermal cells cover a maximum area and growth therefore of trichomes is reduced to a subnormal number.

Where modifications are known in other plant parts, such as in the stem of tobacco, fruits of tomato, pepper and bean, they occur in the chlorenchyma and are similar in nature to those of the leaf.

In the floral parts of tobacco, petunia and sweet pea, white areas are hypoplastic.

No modifications in vascular tissues comparable with those in potato Leafroll, or in sugar-cane and corn mosaic have been found. There may be reduction in size but this is part of the general dwarfing of the plant.

"Streak" or "Leaf-drop" of potato is discussed in relation to mosaic although it is not shown that this trouble is definitely a stage of mosaic. There is, however, a distinct parallel between the lesions in petiole and stem and those occurring in sugar-cane and corn, but the sudden wilting of some of the leaves in the case of potato is not paralleled in diseased corn or sugar-cane.

The various theories concerning the etiology of mosaic are discussed and certain results obtained by the writer are mentioned. The presence in diseased tobacco tissues of minute bodies 0.3 microns long is noted.

The experiments of Lodewijks concerning the effect of colored light on tobacco mosaic were repeated under more exact conditions. In one group of tests the effect of definite group wave-lengths of light on freshly inoculated plants was tested, and in the other a series of tests was conducted to ascertain the effect of colored light on the virus.

It was found that blue light is not remedial in its effect, but that the absence of marked symptoms is due to modified growth conditions. When the plants are maintained at a temperature around 75° F. symptoms become manifest but not marked because of the checking of growth under the blue light. On removal into ordinary daylight new leaves are markedly affected. It is believed that the higher temperature inside the hoods which must have occurred in the experiments of Lodewijks, as is indicated in my own, would account for the masking of symptoms even if growth were fairly well maintained.

It was found that green light enhanced the symptoms somewhat and in the case of uranium glass excessive dwarfing occurred while uviol glass advanced blossoming by a week or more.

Extracted tobacco sap from diseased plants was diluted and half filtered through paper only while the other half was passed under slightly reduced pressure through a Chamberland F filter. The effect of red, green and blue lights, sunlight, darkness, and freezing for an extented period was tested on each filtrate. Extended freezing reduced the virulence of the filtrate. There was little difference between that kept under red and blue light. Darkness and green light were approximately similar and the paper filtrate in both cases was more infective than that which was passed through the porcelain candle.

Transmission of mosaic by *Macrosiphum pisi Kalt* is shown to occur between *Trifolium pratense, T. hybridum, T. repens, T. incarnatum,* and *Medicago lupulina.* In one instance the same aphids infected a plant of *Medicago sativa* from *T. pratense.* Raspberry mosaic is also transmitted by aphids, probably *Aphis rubiphila.*

Cross inoculations using expressed juice from diseased plants were successful between *Trifolium pratense, T. Hybridum, T. repens, T. incarantum* and *Medicago lupulina.* Negative results were obtained in cross-inoculations between *Pisum sativum* or *Phaseolus vulgaris* and the clover's except in the case of one plant of *T. hybridum* which was infected from *P. sativum.*

Seed inheritance of mosaic occurs in *Pisum sativum, Trifolium pratense, T. hybridum* and *Melilotus alba.*

Positive evidence of seed inheritance of mosaic is available for *Hippeastrum sp.* and negative in the case of Raspberry.

ACKNOWLEDGMENTS

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ILLUSTRATIONS

PLATE I

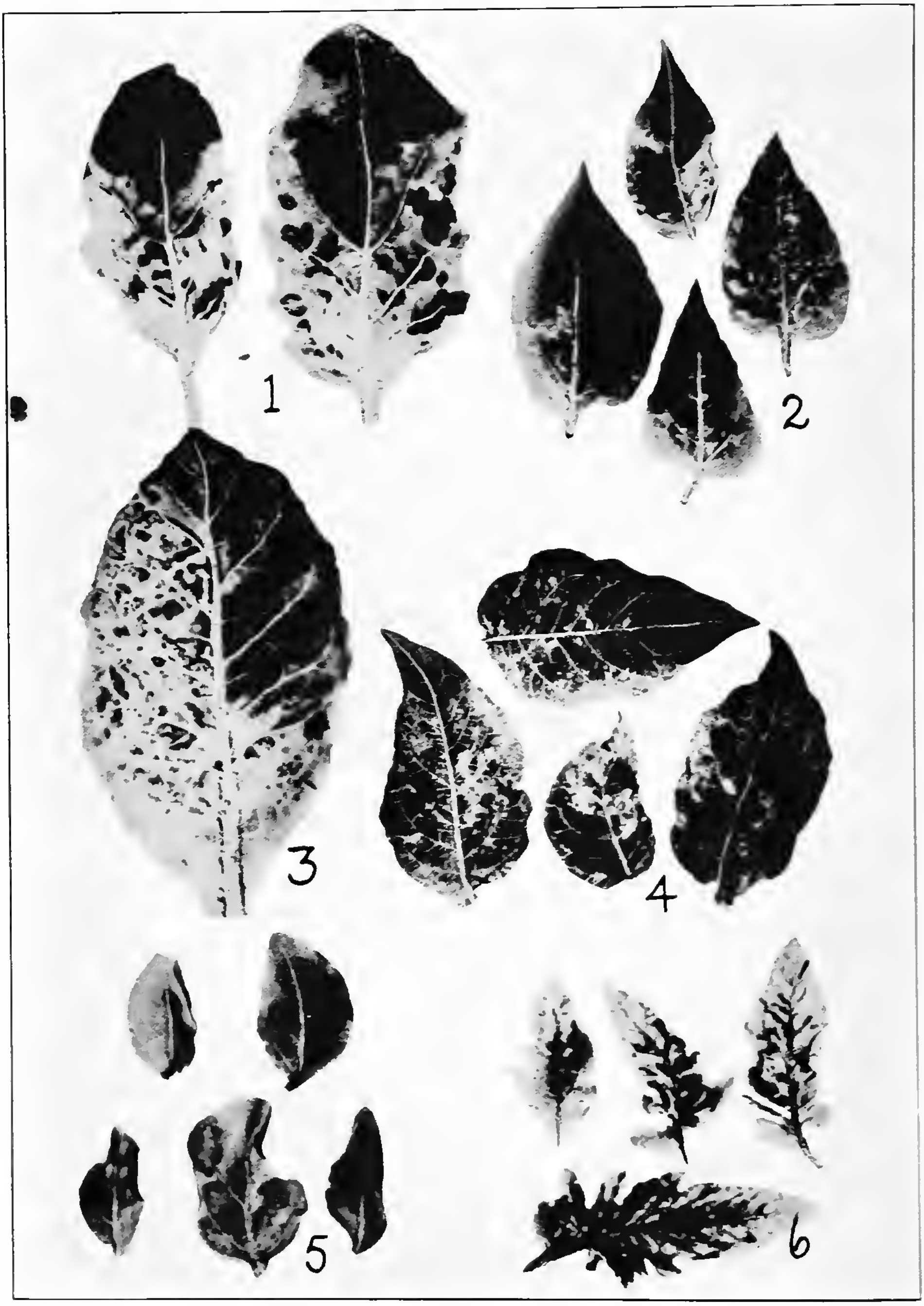


PLATE I

Fig. 1

Black henbane, showing savoying and strong color differentiation.

Fig. 2

Pepper mosaic.

Fig. 3

Tobacco mosaic.

Fig. 4

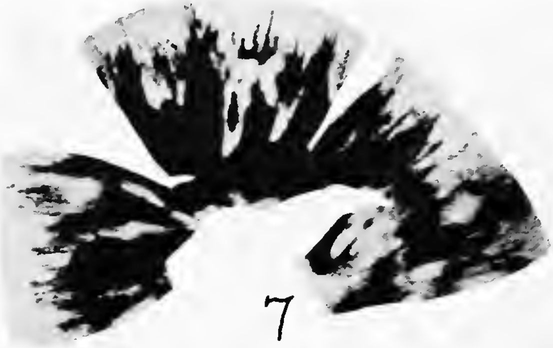
Potato mosaic.

Fig. 5

Petunia, showing excessive bulging of darker parts.

Fig. 6

Tomato leaflets with well marked differentiation. In such leaflets hypoplasia occurs.



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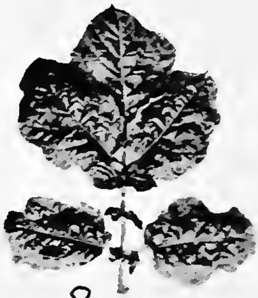

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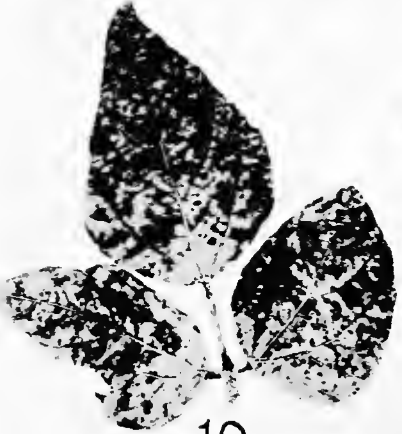

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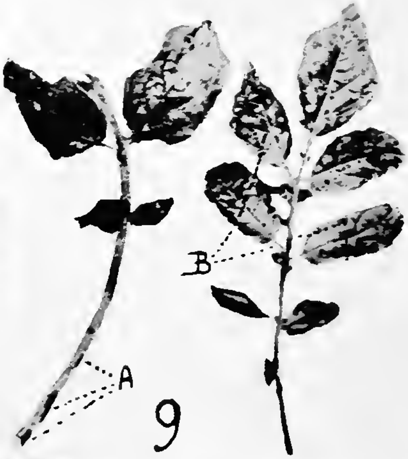

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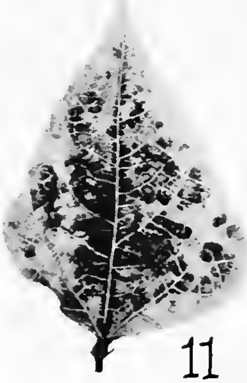

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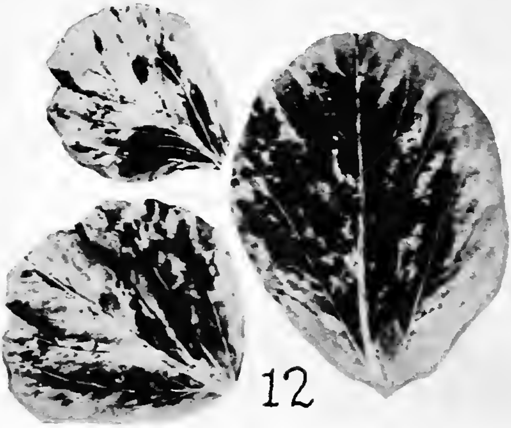

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PLATE II

Fig. 7

Contact photograph of upper part of the ovary wall of mottled tomato.

Fig. 8

Curled type of potato mosaic.

Fig. 9

Potato leaves from plant affected with "streak" and "leaf drop". Note streaks of necrotic tissue at A and patches at B.

Fig. 10

Leaf of marrow bean showing speckled type of mottling.

Fig. 11

Leaf of pea bean showing blistered type.

Fig. 12

Mosaic of Windsor bean.

PLATE III

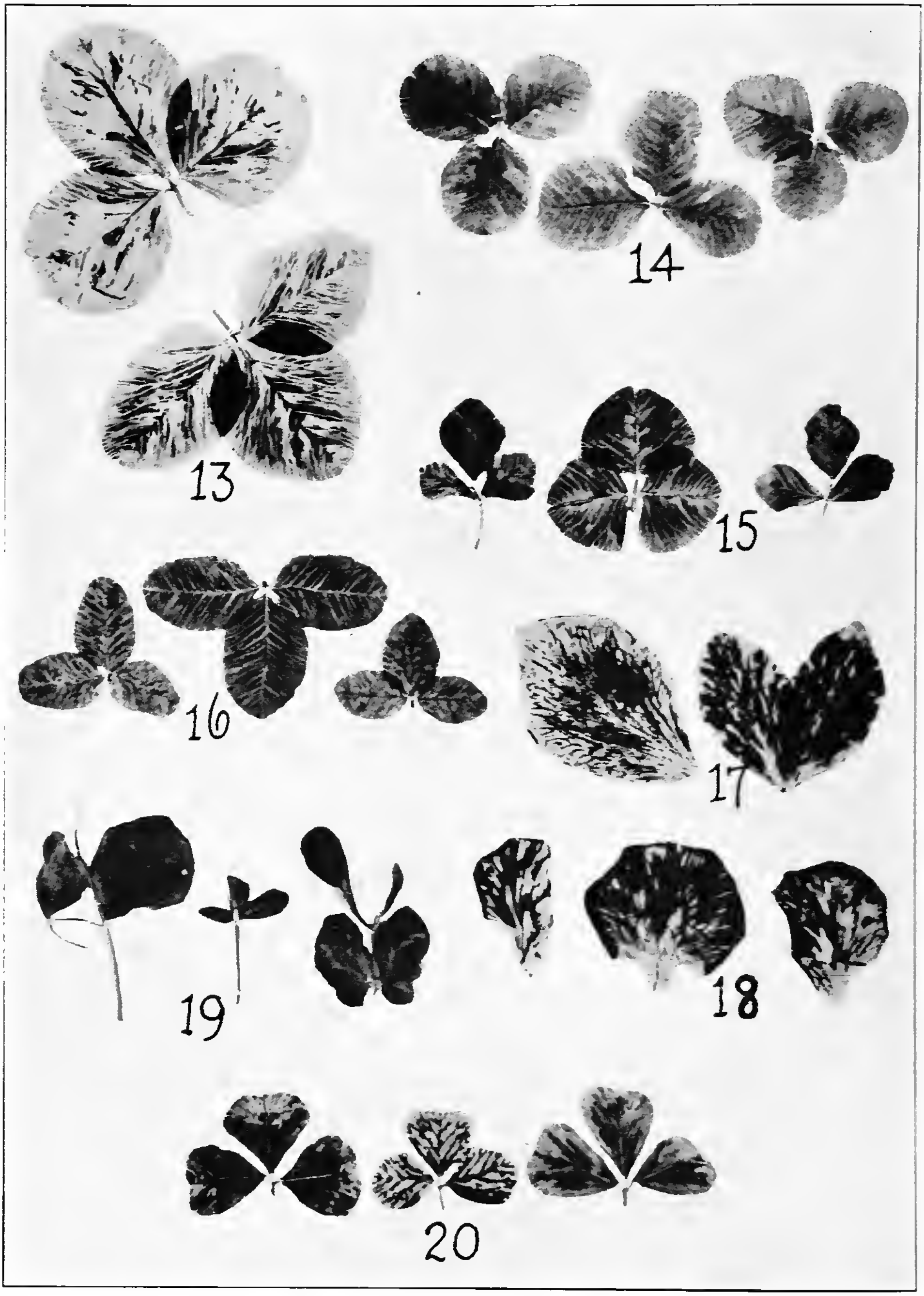

PLATE III

Fig. 13

Red clover mosaic.

Fig. 14

White or Dutch Clover mosaic.

Fig. 15

Trefoil clover mosaic.

Fig. 16

Alsike clover. Note fine speckling type of mosaic.

Fig. 17

Mosaic-diseased sweet pea leaflets.

Fig. 18

Floral parts of red purple sweet pea showing mottling.

Fig. 19

Leaves of Canada field pea illustrating the types of growth as a result of nutritional disturbances in mosaic-diseased plants.

Fig. 20

Crimson clover.

PLATE IV

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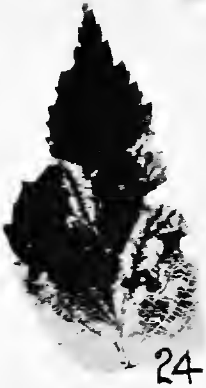
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PLATE IV

Fig. 21

Tip of leaf of *Hydrangea* showing effect of aphids. (Not a true mosaic).

Fig. 22

Leaf of *Abutilon* illustrating the type of variegation.

Fig. 23

Leaflets of Canada field pea showing crinkling and mottling. (This is the only photograph of a mosaic-diseased specimen not taken by direct contact.)

Fig. 24

Raspberry mosaic.

Fig. 25

Part of leaf of *Hippeastrum sp.* with mosaic. This leaf was too thick to give clear differentiation.

Fig. 26

Parts of sugar-cane leaves with mosaic.

PLATE V

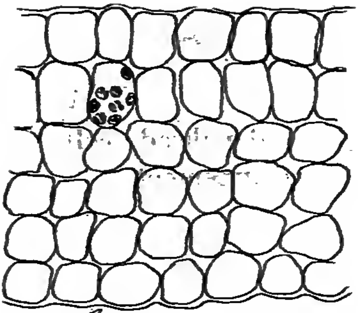
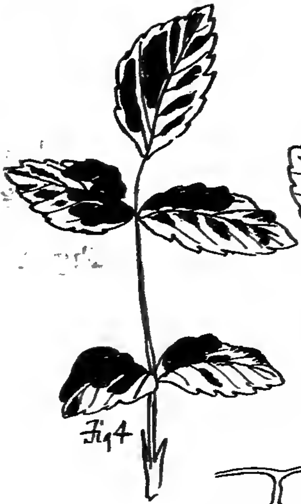


Fig. 1



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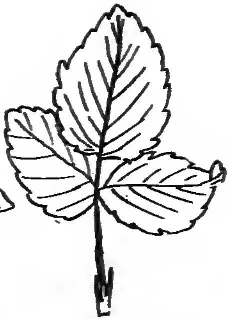

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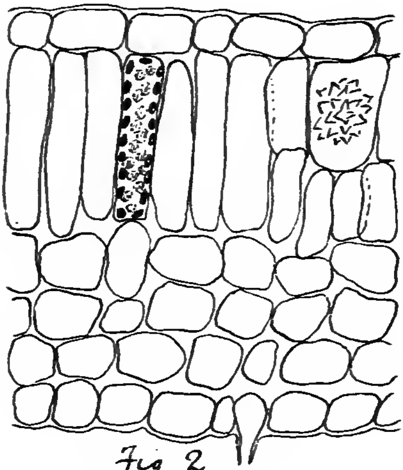

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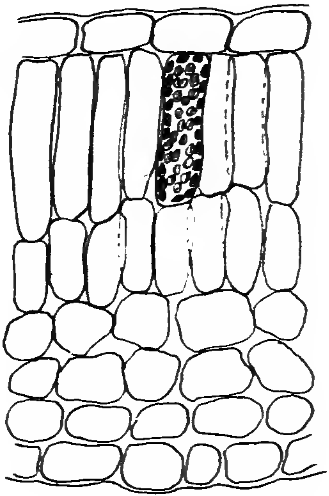

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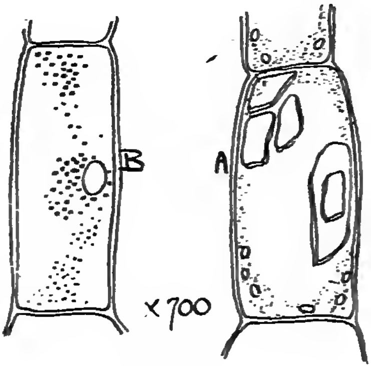

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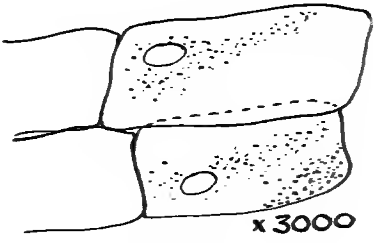


Fig. 7

PLATE V

Fig. 1

Hypoplastic area in raspberry showing cuboidal palisade cells, scarcity of chloroplasts and regular cell arrangement.

Fig. 2

Section through dark green area of the ordinary type in diseased raspberry.

Fig. 3

Section through excessively corrugated dark green area in diseased raspberry. Note hypertrophy and hyperplasia in palisade tissue.

Fig. 4

Drawing of typical leaf of raspberry with mosaic. Note excessive corrugation of dark green areas and arrangement of leaflets.

Fig. 5

Drawing of typical leaf of raspberry with "yellows or curl"

Fig. 6

Drawing of trichome illustrating lamellae at A and hyaline bodies at B.

Fig. 7

Minute bodies in phloem parenchyma cell, stained with Giemsa.

PLATE VI

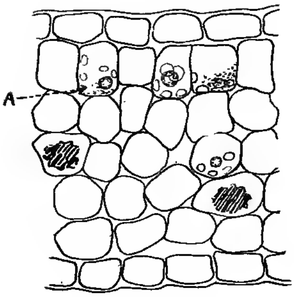
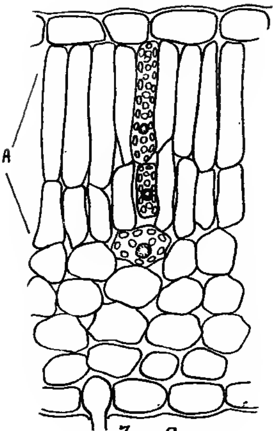


Fig. 8



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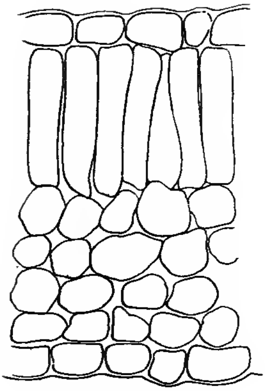

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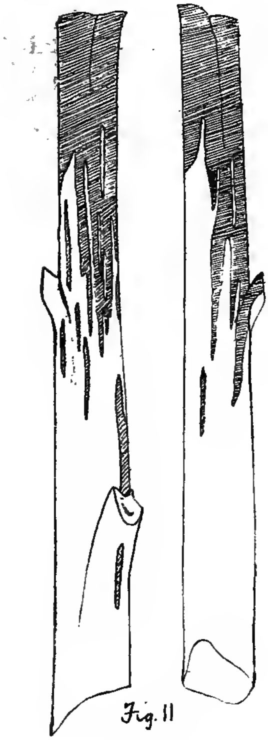

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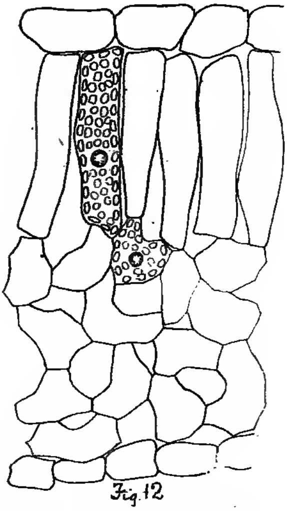

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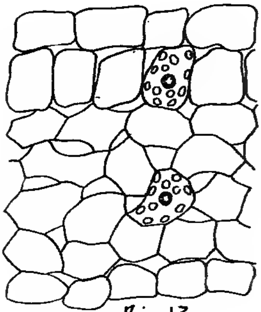

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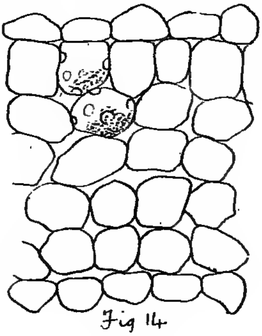


Fig 14

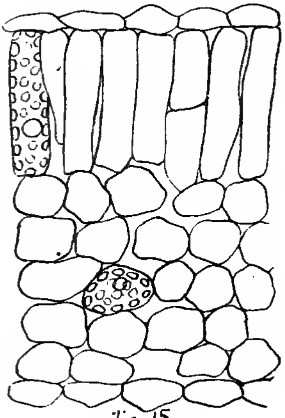


Fig 15

PLATE VI

Fig. 8

Section through hypoplastic area of tobacco leaf. At A note disorganized chloroplasts.

Fig. 9

Section through dark green area showing hypertrophy of palisade tissue in tobacco.

Fig. 10

Section through ordinary dark green tissue of tobacco leaf.

Fig. 11

Drawings to show dark green stripes on diseased tobacco stems.

Fig. 12

Section through dark green areas of *Vicia faba* leaf with mosaic.

Fig. 13

Section through hypoplastic area of *Vicia faba* leaf with mosaic.

Fig. 14

Section through light colored area in red clover leaflet. Observe the disorganized chloroplasts.

Fig. 15

Section through dark green area of leaflet of red clover.

PLATE VII

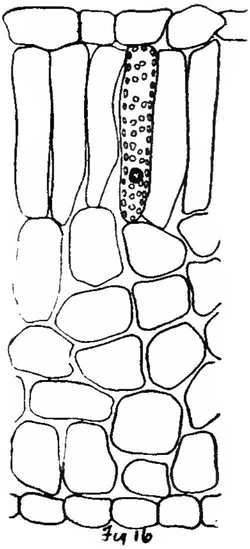
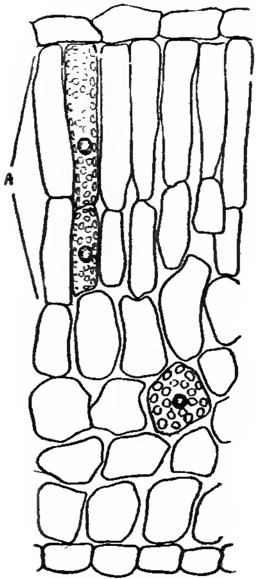


Fig 16



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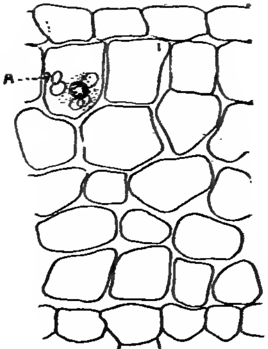

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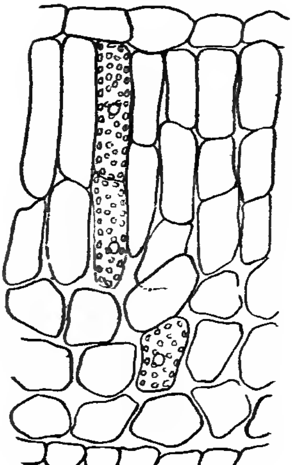
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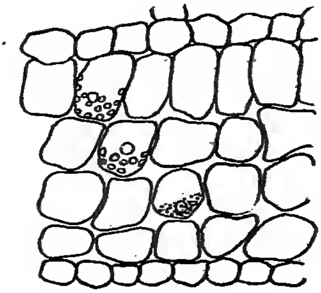
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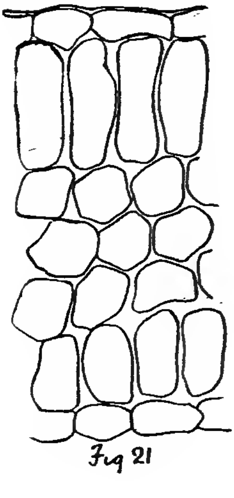
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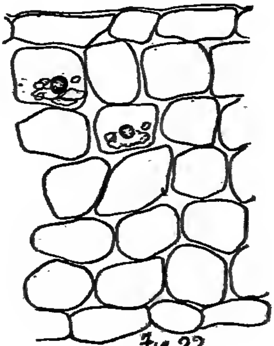
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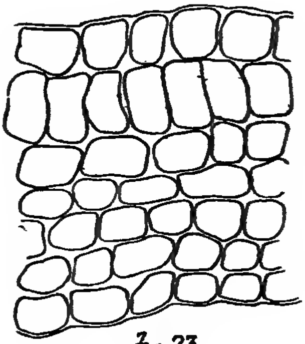


Fig. 23

PLATE VII

Fig. 16

Section through ordinary dark green tissue of *Hyoscyamus* leaf with mosaic.

Fig. 17

Hypertrophy in *Hyoscyamus* leaf heavily infected when young.

Fig. 18

Section through hypoplastic area of diseased *Hyoscyamus* leaf showing cuboidal palisade cells and disorganized chloroplasts.

Fig. 19

Section through diseased petunia leaf showing hypertrophy in palisade tissue.

Fig. 20

Hypoplastic area of petunia.

Fig. 21

Section through dark green area of sweet pea.

Fig. 22

Section of hypoplastic tissues of sweet pea.

Fig. 23

Section through diseased tomato leaf showing cuboidal cells of the palisade tissue.

PLATE VIII

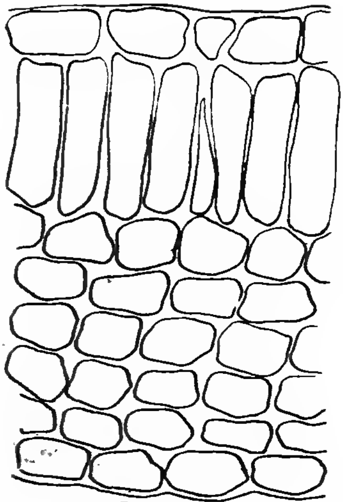


Fig 24

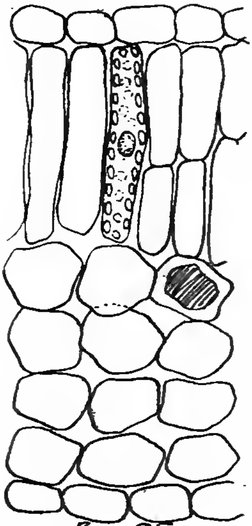
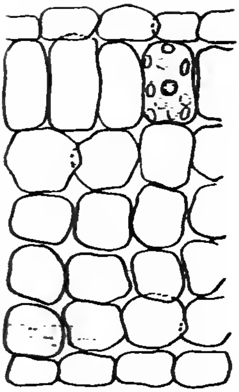


Fig 25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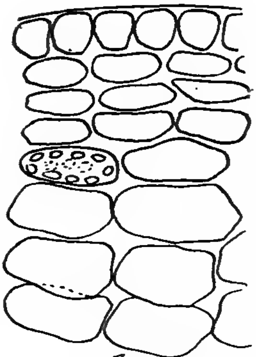
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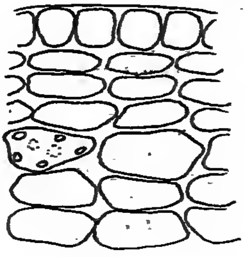
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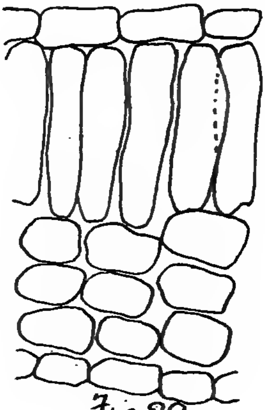
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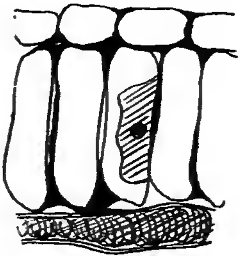
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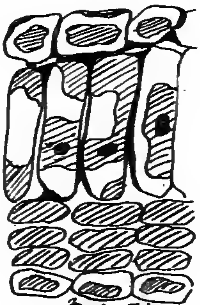
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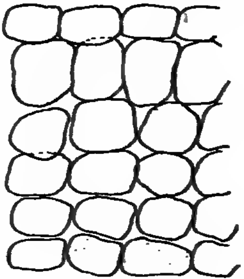
Fig. 30

PLATE VIII

Fig. 24

Section through the dark area of a tomato leaf with mosaic.

Fig. 25

Section through the dark area of a leaf of pepper with mosaic.

Fig. 26

Section through the hypoplastic area in a pepper leaf.

Fig. 27

Section of a dark patch on the fruit of a tomato with mosaic.

Fig. 28

Section through a light-colored area of the same fruit.

Fig. 29

Potato leaf section through a dark area.

Fig. 30

Section through a light green area of the same leaf.

Fig. 31

Section through a badly affected area of potato leaf with streak. Note the collapsed cells.

Fig. 32

Section through another area in the same leaf showing the beginnings of necrosis. Note the necrosis of the cell walls indicated by heavy shading.